FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION:

NADA Number: 140-863

Sponsor: Elanco Animal Health
A Division of Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

Generic Name: Ractopamine hydrochloride

Trade Name: Paylean® (a registered trademark of Elanco Animal Health)

Marketing Status: Over the Counter (OTC)

II. INDICATIONS FOR USE:

For increased rate of weight gain, improved feed efficiency, and increased carcass leanness in finishing swine fed a complete ration containing at least 16% crude protein from 150 lb (68 kg) to 240 lb (109 kg) body weight.

III. DOSAGE:

a. Dosage Form: Paylean® Type A Medicated Article is available as a 25-pound bag containing 9 grams of ractopamine hydrochloride per pound.

b. Route of Administration: Oral, via the feed

c. Recommended Dosage:

Ractopamine hydrochloride should be fed at a concentration of 4.5 g of ractopamine hydrochloride per ton of complete feed (5 ppm) for increased rate of weight gain, improved feed efficiency, and increased carcass leanness in a ration containing at least 16% crude protein from 150 lb (68 kg) to 240 lb (109 kg) body weight.

Ractopamine hydrochloride should be fed at a concentration of 4.5 g to 18 g of ractopamine hydrochloride per ton of complete feed (5 ppm to 20 ppm) for improved feed efficiency and increased carcass leanness in a ration containing at least 16% crude protein from 150 lb (68 kg) to 240 lb (109 kg) body weight.

IV. EFFECTIVENESS:

Four dose titration/confirmation clinical effectiveness trials for growth performance (weight gain and feed efficiency), and three dose titration/confirmation clinical effectiveness trials for carcass leanness parameters, were reported. The data from these trials provided substantial evidence of effectiveness that Paylean® Type A Medicated Article, as a production drug, will increase the rate of weight gain, improve feed efficiency, and increase carcass leanness in finishing swine fed a complete ration containing at least 16% crude protein from 150 lb (68 kg) to 240 lb (109 kg) body weight.

IV.A. Dose Titration/Confirmation Clinical Effectiveness Trials For Performance Parameters

The objective of these trials was to determine the clinical effectiveness and efficacious dose range of ractopamine hydrochloride (RACT) for improving the rate of body weight gain and feed efficiency in finishing swine when fed a diet containing 16% crude protein from 150 lb to 240 lb. The trials were conducted with similar protocols so the results of each could be pooled and summarized for evaluation. Data were pooled from four adequate and well-controlled trials involving 583 crossbred finishing pigs conducted in various swine production areas of the United States. Trial facilities were typical of those used by commercial swine operations in each location. The trials were designed as "equal time" trials in which the trial period was terminated when the average weight of the heaviest pen (within a replicate) was approximately 235 lb. Barrows and gilts were fed and penned separately in each trial.

Each trial consisted of seven treatments:

1) a conventional 13% crude protein diet with no RACT (control)
2) a conventional 13% crude protein diet with 18 g/ton (20 ppm) RACT
3) a 16% crude protein diet with no RACT (control)
4) a 16% crude protein diet with 4.5 g/ton (5 ppm) RACT
5) a 16% crude protein diet with 9 g/ton (10 ppm) RACT
6) a 16% crude protein diet with 13 g/ton (15 ppm) RACT
7) a 16% crude protein diet with 18 g/ton (20 ppm) RACT

In each trial, seven treatment groups were replicated four times with two blocks of barrows and two blocks of gilts in a randomized complete block design. While seven treatments were used in the trials, only the five treatments used in a 16% crude protein diet were used to determine the effective dose range of RACT for improving rate of weight gain and feed efficiency. Blocks were environmental locations within the finishing facilities. Within sexes, blocks consisted of contiguous pens. Initially, individual pigs weighed 150 lb ± 5.0% with the average pen weight being 150 lb ± 2.5%.

Experimental feeds were prepared from uniform 13% or 16% crude protein finishing diets using feed ingredients and formulations common to the production practices in those regions. Experimental feeds and water were offered *ad libitum* throughout the trials.

Feeding or treatment duration ranged from 35 to 48 days with feeding length consistent within each block. Nutritional composition and appropriate drug concentration of the experimental feeds were verified by appropriate analyses. Treatments were blinded to all trial site personnel involved in the trials.

IV.A.1. Pooled Data From Four Effectiveness Trials

Data from the four trials were pooled and analyzed. Variables of interest were average daily weight gain, average daily feed intake, feed to gain ratio and gain to feed ratio. Variables were statistically evaluated using mixed model methodology. Comparisons between controls and different levels of RACT in the 16% crude protein diets were made to establish the lowest effective dose for the dose range for average daily weight gain and improved feed efficiency. Appropriate comparisons for the combined 13% and 16% crude protein data were made to evaluate certain safety aspects of the study.

The least squares means from the pooled analysis of the performance variables are shown in Table IV.1. Contrasts based on the pooled results for rate of weight gain showed that each non-zero level of RACT in a 16% crude protein diet was significantly different from controls ($P \leq 0.06$). Modeling indicated that the rate of weight gain response was the same for all levels of RACT [from 4.5 g/ton (5 ppm) to 18 g/ton (20 ppm)]. Feed efficiency (F/G) responses for each level of RACT in 16% crude protein diets were significantly different from control ($P \leq 0.0001$). Feed efficiency response was best modeled by two sloping lines, indicating decreasing feed to gain ration over the range of 4.5 g/ton (5 ppm) to 18 g/ton (20 ppm) RACT. Linear contrasts showed average daily feed intake to be significantly different ($P \leq 0.04$) from control animals to animals treated with 13 g/ton (15 ppm) and 18 g/ton (20 ppm) RACT.

No adverse effects were observed for any treatments. In the 13% crude protein, 20 ppm treatment, the reduced growth performance response compared to the 16% crude protein diets may be partially attributed to insufficient levels of crude protein and hence amino acids (primarily available lysine).

These data show that RACT fed in a 16% crude protein diet is effective for increasing rate of weight gain and improving feed efficiency.

Table 1. Pooled Four Trial Summary of Performance Variables - Ractopamine Hydrochloride Performance Trials

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)[b]	1.81	1.95	1.92	1.90	1.96	1.75	1.78
SE (lb./head/day)[c]	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Average Daily Feed Intake (lb)[b]	6.30	6.27	6.09	5.89	6.02	6.14	5.99
SE (lb./head/day)[c]	0.21	0.21	0.21	0.21	0.21	0.22	0.21
Feed to Gain Ratio[b]	3.51	3.22	3.18	3.13	3.08	3.51	3.40
SE [c]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Gain to Feed Ratio[b]	0.287	0.311	0.316	0.322	0.326	0.286	0.296
SE [c]	0.009	0.009	0.009	0.009	0.009	0.009	0.009

[a]crude protein (%)-ractopamine (ppm)
[b]least squares means
[c]standard error

IV.A.2. Individual Effectiveness Trials:

Trial T4V379203. A dose titration trial was conducted in barrows and gilts fed separately by Joan H. Eisemann, Ph.D., North Carolina State University, Raleigh, NC. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 35 pigs per block and five pigs per pen. A total of 139 animals were used in the trial. Corn and soybean meal-based diets typical of the region were used in the trial. Feeding duration ranged from 35 to 44 days with feeding length consistent within each block. Three animals were removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V379203

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	1.95	2.14	2.14	2.16	2.22	1.88	2.00
SD[b]	0.17	0.26	0.25	0.21	0.18	0.24	0.22
Average Daily Feed Intake (lb)	6.44	6.52	6.34	6.12	6.48	6.07	6.20
SD[b]	0.71	0.58	0.84	0.45	0.48	0.65	0.58
Feed to Gain Ratio	3.30	3.06	2.96	2.84	2.93	3.24	3.11
SD[b]	0.20	0.14	0.15	0.07	0.08	0.11	0.05
Gain to Feed Ratio	0.304	0.327	0.338	0.352	0.342	0.309	0.322
SD[b]	0.02	0.01	0.02	0.01	0.01	0.01	0.01

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

Trial T4V189204. A dose titration trial was conducted in barrows and gilts fed separately by Tilford R. Cline, Ph.D., Purdue University, West Lafayette, IN. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 33-35 pigs per block and four or five pigs per pen. A total of 136 animals were used in the trial. Corn and soybean meal-based diets typical of the region were used in the trial. Feeding duration ranged from 40 to 48 days with feeding length consistent within each block. One animal was removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V189204

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	1.66	1.74	1.64	1.79	1.66	1.64	1.52
SD[b]	0.13	0.16	0.19	0.24	0.17	0.10	0.12
Average Daily Feed Intake (lb)	5.80	5.59	5.04	5.45	5.08	5.76	5.27
SD[b]	0.41	0.49	0.43	0.58	0.25	0.55	0.33
Feed to Gain Ratio	3.49	3.21	3.10	3.06	3.07	3.52	3.47
SD[b]	0.16	0.06	0.29	0.15	0.16	0.19	0.08
Gain to Feed Ratio	0.287	0.312	0.325	0.328	0.326	0.285	0.288
SD[b]	0.01	0.01	0.03	0.02	0.02	0.02	0.01

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

Trial T4V319205. A dose titration trial was conducted in barrows and gilts fed separately by Austin J. Lewis, Ph.D. and Phillip S. Miller, University of Nebraska, Lincoln, NE. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 42 pigs per block and six pigs per pen. A total of 168 animals were used in the trial. Corn and soybean-meal based diets typical of the region were used in the trial. Feeding duration ranged from 37 to 45 days with feeding length consistent within each block. One animal was removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V319205

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	1.89	2.08	2.03	1.97	2.02	1.85	2.01
SD[b]	0.16	0.13	0.10	0.26	0.08	0.13	0.12
Average Daily Feed Intake (lb)	6.51	6.66	6.41	6.18	6.21	6.42	6.52
SD[b]	0.40	0.62	0.41	0.67	0.52	0.34	0.29
Feed to Gain Ratio	3.45	3.19	3.16	3.15	3.07	3.48	3.24
SD[b]	0.28	0.13	0.12	0.11	0.14	0.11	0.11
Gain to Feed Ratio	0.291	0.314	0.317	0.317	0.326	0.287	0.309
SD[b]	0.02	0.01	0.01	0.01	0.01	0.01	0.01

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

Trial T4V179206. A dose titration trial was conducted in barrows and gilts fed separately by Robert A. Easter, Ph.D. and Leroy Biehl, DVM, MS, University of Illinois, Champaign-Urbana, IL. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 35 pigs per block and five pigs per pen. A total of 140 animals were used in the trial. Corn and soybean meal-based diets typical of the region were used in the trial. Feeding duration ranged from 39 to 45 days with feeding length consistent within each block. Twelve animals were removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V179206

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	1.72	1.84	1.87	1.69	1.92	1.68	1.60
SD[b]	0.19	0.15	0.18	0.28	0.15	0.18	0.31
Average Daily Feed Intake (lb)	6.47	6.30	6.57	5.80	6.18	6.42	5.95
SD[b]	0.47	0.14	0.32	0.49	0.56	0.58	0.76
Feed to Gain Ratio	3.78	3.43	3.52	3.47	3.23	3.83	3.76
SD[b]	0.18	0.24	0.19	0.36	0.31	0.14	0.30
Gain to Feed Ratio	0.265	0.293	0.285	0.290	0.312	0.262	0.267
SD[b]	0.01	0.02	0.02	0.03	0.03	0.01	0.02

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

IV.B. Dose Titration/Confirmation Clinical Effectiveness Trials For Carcass Leanness Parameters

The objective of the trials was to determine the clinical effectiveness and efficacious dose range of RACT for increasing carcass leanness measured by changes in percentage carcass chemical lean of finishing swine when fed from 150 to 240 lb including a 5-day preslaughter withdrawal period. Data were pooled from three adequate and well-controlled trials involving crossbred finishing pigs conducted in various swine production areas of the United States. Live phase investigators, trial locations and number of pigs involved are summarized by trial number in a Table under section IV.D., Corroborative studies, of this summary (*vide infra*). Trial facilities were typical of those used by commercial swine operations in each location. Live phase performance is addressed under section IV.D., Corroborative studies, of this summary.

The carcass phase investigators and slaughter facilities are listed in Table IV.2. Animals were slaughtered using typical slaughter procedures under USDA inspection.

Table IV.2. Investigators and Slaughter Facilities - Ractopamine Hydrochloride Carcass Trials

Trial	Investigator and Slaughter Facility
Nebraska (T4V319207)	Roger Mandigo, Ph.D. University of Nebraska, Meat Laboratory Lincoln, NE
Illinois (T4V179208)	Floyd K. McKeith, Ph.D. University of Illinois, Meat Laboratory Champaign-Urbana, IL
Iowa (T4V199209)	Ken Prusa, Ph.D. Iowa State University, Meat Laboratory Ames, IA

Animals from the performance trials were used as the pool of animals for selecting at least one and preferably two animals per pen for slaughter and determining changes in carcass leanness due to RACT as measured by percentage chemical lean. The trials were designed as "equal weight" trials in which the treatment period was terminated when each pen weighed approximately 235 lb. At that time, the pen was placed on a 5-day preslaughter withdrawal period.

Each trial consisted of seven treatments:

1) a conventional 13% crude protein diet with no RACT (control)
2) a conventional 13% crude protein diet 18 g/ton (20 ppm) RACT
3) a 16% crude protein diet with no RACT (control)
4) a 16% crude protein diet with 4.5 g/ton (5 ppm) RACT
5) a 16% crude protein diet with 9 g/ton (10 ppm) RACT
6) a 16% crude protein diet with 13 g/ton (15 ppm) RACT
7) a 16% crude protein diet with 18 g/ton (20 ppm) RACT

Treatments were blinded to all meat laboratory site personnel involved in the trials. In each trial, seven treatment groups were replicated four times with two blocks of barrows and two blocks of gilts in a randomized complete block design. While seven treatments were used in the trials, only the five treatments with 16% crude protein were used to determine the effective dose range of RACT for improvement in carcass leanness as measured by percentage chemical lean. Blocks were environmental locations within the finishing facilities. Within sexes, blocks consisted of contiguous pens. Initially, individual pigs weighed 150 lb ± 5.0% with the average pen weight being 150 lb ± 2.5%. At the end of the withdrawal period at least one and preferably two animals per pen

weighing between 234 to 246 lb were selected for slaughter and determining carcass composition.

Standard pork carcass measurements described in the National Pork Producers Council publication: *Procedures to Evaluate Market Hogs*, 3rd Edition, 1991 were collected following slaughter.

Increased carcass leanness was evaluated by measuring changes in the percentage of carcass chemical lean of treatments as compared to controls. The procedure consists of:

> Physical separation of the carcass side into bone, soft tissue and skin; followed by weighing of bone, soft tissue and skin and determination of chemical fat, protein, ash, and moisture in a composite, representative sample of the "boned-out" soft tissue. A.O.A.C. analysis of the representative sample for chemical fat, protein, ash and moisture was conducted at an independent laboratory.
>
> For this procedure: Weight of Lean = Weight of Chemical Lean = (weight of soft tissue) minus (weight of fat + weight of ash + weight of moisture).

Data from the three trials were pooled and analyzed. Variables of interest for carcass leanness were percentage chemical lean and chemical lean weight of the carcass. The carcass leanness variables were statistically evaluated using either general linear models or mixed model methodology. Comparisons between 16% crude protein controls and different levels of RACT in the 16% crude protein diets were made to establish a dose range for improvement in carcass leanness as measured by percentage carcass leanness. Appropriate comparisons for the combined 13% and 16% crude protein data were made to evaluate certain safety aspects of the study.

The results of the statistical analysis of the 16% crude protein, data are shown in Table IV.3. Pooled results showed that carcass leanness as measured by percentage chemical lean is adequately modeled by a simple linear regression, indicating increasing carcass leanness in the range of 4.5 g/ton (5 ppm) to 18 g/ton (20 ppm) of RACT.

Table IV.3. Summary of Carcass Leanness Variables from Combined 13 and 16 Percent Protein Data - Ractopamine Carcass Trials

	Treatment[a]						
Carcass Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Chemical Lean (%)[b]	12.05	12.18	12.61	12.65	12.86	11.63	12.02
SE[c]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chemical Lean Wt (lb)[b]	10.26	10.34	10.99	10.95	11.11	9.95	10.44
SE[c]	0.19	0.19	0.19	0.19	0.19	0.20	0.20

[a]crude protein (%)-ractopamine (ppm)
[b]least square means
[c]standard error

No adverse effects were observed for any treatments. In the 13% crude protein, 18 g/ton (20 ppm) treatment however, the reduced response on carcass leanness compared to the 16-0 treatment may be partially attributed to insufficient levels of crude protein and hence amino acids (primarily available lysine).

One hundred fifty three (153) pigs weighing between 234 and 246 lb were included in the carcass analyses from these trials. Of these pigs, one hundred and eleven (111) were fed RACT at levels of 5, 10, 15 and 20 ppm. No treatment related adverse effects related to carcass measurements and carcass composition were observed during the trials. These data show that RACT fed in a 16% crude protein diet is effective for increasing carcass leanness when used in the range of 5 to 20 ppm.

Additional variables collected or calculated for the label statements that may be important to the commercial industry were:

1) Percent Chemical Fat
2) Fat Depth 10th Rib
3) Backfat Thickness Last Rib
4) Loin Eye Area
5) Average Daily Lean Gain
6) Efficiency of Lean Gain
7) Dressing Percent

The results of the statistical analysis of the 16% crude protein data are shown in Table IV.4. Statistical P values from appropriate comparisons are shown in Table IV.5.

Table IV.4. Summary of Least Squares Means for Carcass Variables of Commercial Interest from Combined 13 and 16 Percent Protein Data - Ractopamine Carcass Trials

	Treatment[a]						
Carcass Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Carcass Fat (%)[b]	29.0	28.9	26.7	27.2	26.5	30.3	29.3
SE[c]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Fat Depth 10th Rib (in)[b]	1.14	1.14	1.05	1.12	1.05	1.20	1.19
SE[c]	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Backfat Thickness Last Rib (in)[b]	1.00	1.06	1.02	1.02	1.03	1.02	1.09
SE[c]	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Loin Eye Area (in^2)[b]	5.19	5.45	5.76	5.50	5.71	5.08	5.37
SE[c]	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Average Daily Lean Gain (lb)[b]	0.163	0.179	0.213	0.209	0.220	0.153	0.181
SE[c]	0.009	0.009	0.009	0.009	0.009	0.009	0.009
Efficiency of Lean Gain[b]	0.024	0.028	0.033	0.033	0.035	0.023	0.028
SE[c]	0.001	0.001	0.001	0.001	0.001	0.002	0.002

Dressing Percent[b]	72.30	73.09	74.25	73.88	74.29	73.00	74.38
SE[c]	0.41	0.41	0.41	0.41	0.41	0.42	0.42

[a]crude protein (%)-ractopamine (ppm)
[b]least square means
[c]standard error

Table IV.5. Summary of Statistical P Values for Carcass Variables of Commercial Interest from Combined 13 and 16 Percent Protein Data - Ractopamine Carcass Trials

Carcass Variable	Comparison - P value Treatment[a]			
	16-5 vs. 16-20	16-0 vs. 16-10	16-0 vs. 16-20	13-0 vs. 13-20
Carcass Fat (%)	0.92	0.03	0.02	0.38
Fat Depth 10th Rib	0.92	0.17	0.14	0.90
Backfat Thickness Last Rib	0.16	0.71	0.45	0.17
Loin Eye Area	0.13	0.002	0.005	0.14
Average Daily Lean Gain	0.15	0.0001	0.0000	0.02
Efficiency of Lean Gain	0.09	0.0000	0.0000	0.02
Dressing Percent	0.06	0.0000	0.0000	0.001

[a]crude protein (%)-ractopamine (ppm)

IV.C. Dose Titration/Confirmation Clinical Effectiveness Trials For Sensory Parameters

The objective of this study (T4V179211) was to determine the effect of RACT on the trained panel sensory evaluation properties of cooked fresh pork center loin roasts and cured semi-boneless ham. Floyd McKeith, Ph.D. and Doug Roth, M.S. were co-investigators for this study that was conducted at the University of Illinois Meat Laboratory, 1503 South Maryland Drive, Urbana, IL 61801.

The pork loin roasts (*longissimus dorsi* muscle) and cured semi-boneless ham (*semimembranosus* muscle) samples evaluated were obtained from 4 clinical trials described above (T4V179206, T4V319207, T4V179208 and T4V199209). The experimental design was a randomized complete block. The treatments evaluated included: five (5) levels of ractopamine (0, 5, 10, 15 and 20 ppm) in a 16% crude protein diet and two (2) levels (0 and 20 ppm) in a conventional 13% crude protein diet. The loin and ham samples were removed from two animals per pen for each treatment from pigs slaughtered at the completion of the live phase of the trials. Sensory investigators were blinded to treatments and codes.

All samples were collected following normal industry slaughter and chilling processes. Samples were identified by animal number and trial number. Conduct of the sensory panels and preparation of samples was consistent with the procedures described in the *Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat*, American Meat Science Association. Sensory evaluation was conducted by trained panelists. Sensory panelists were trained to clearly distinguish differing amounts of juiciness, tenderness, flavor, and off-flavors. This training enables differentiation of the variables in increments more discreet and refined than the typical consumer could detect. Panelists were trained and used a 15 cm semi-structured line

scale to critically evaluate and detect small differences in sensory traits the consuming public typically does not observe.

The sensory variables evaluated were:

1) juiciness
2) tenderness
3) flavor intensity
4) off flavor intensity

The mechanical evaluation for tenderness - Warner-Bratzler shear force was also evaluated.

Semi-boneless hams were manufactured in a manner that mimicked commercial practices. Fresh pork loin roasts were cooked to an internal temperature of approximately 70^{o}C prior to sensory evaluation. Warm pork loin and ham samples were served to the panelists. A minimum of five cores were removed parallel to the direction of the muscle fibers from the slices for Warner-Bratzler shear force measurement.

Sensory evaluation data from the samples collected from the four trials were pooled and analyzed. The five variables of interest were statistically evaluated using either general linear models or mixed model methodology. Comparisons between controls and different levels of RACT in the 16% crude protein diets were made to evaluate the sensory properties of cooked pork loin samples and cured ham samples. Appropriate comparisons for the combined 13% and 16% crude protein data were made to evaluate certain safety aspects of the study.

The results of the sensory panel evaluation of fresh loin roasts from pigs fed a 16% crude protein diet with 0, 5, 10, 15 or 20 ppm RACT showed that the panel could detect no difference in juiciness, tenderness and flavor (Table IV.6.). Analysis of off-flavor data showed the loin roasts from pigs fed 10 ppm or 15 ppm RACT in a 16% crude protein diet had less off-flavor ($P \leq 0.008$) compared to the 16% crude protein controls. However, the magnitude of the differences had no practical meaning from an industry standpoint.

For fresh loin roasts, the Warner-Bratzler shear force measurements showed a trend toward higher values at 10 ppm and 15 ppm RACT. However, only at the 20 ppm RACT level compared to the 16% crude protein controls was a difference ($P \leq 0.01$) detected. No differences were observed in Warner-Bratzler shear force between sexes in this study. Although there were differences in the Warner-Bratzler shear force when controls were compared to the 20 ppm RACT level, the sensory panel did not detect this increase. Therefore it was concluded that no differences in palatability would be detected by the consumer. The cause of the increase in Warner-Bratzler shear may be partially due to an increase in the muscle fiber area.

Cured ham sensory evaluation and Warner-Bratzler shear force of samples from pigs fed the 16% crude protein ractopamine diets showed no differences for juiciness, tenderness, and flavor.

Small sensory differences were observed between animals fed the 16% crude protein diets and the 13% crude protein diets. Differences between diets and sexes may be expected, but the magnitude of differences in pork loin roasts and cured ham from these trials were

of no practical meaning. Some of the differences in juiciness, tenderness, flavor and Warner-Bratzler shear force were expected since pigs fed a 13 percent crude protein diet treatment would be expected to be fatter than pigs fed a 16 percent crude protein treatment. It has been reported that leaner meat is also less juicy.

Many swine nutrition and genetic programs today use feeds with protein contents between 13 and 16 percent crude protein, therefore, the differences observed between animals receiving the 13 and 16 percent crude protein diets in these studies should be similar to those which exist in present day pork production. A survey of composition and palatability traits of fresh pork from the marketplace conducted in 1988 and in 1994 confirms that the results of the present study are well within the normal ranges observed in pork palatability over the past 10 years.

The cured ham evaluated from the pigs in the present series of studies was developed as a "true ham" product, meaning that by USDA standards minimal water was added to the product to complete the curing process. This type of product should show the "worst case effect" of any ractopamine treatment. Other types of ham or sausage products with added water and other ingredients (salt, sugar, etc.) that mask juiciness, tenderness and flavor differences should show no effects due to ractopamine. In evaluating pork quality and palatability it is important to consider that at least 75% (*Meat Facts*, American Meat Institute, 1992) of the pork carcass is manufactured into further processed products. The net effect of processing is to increase the pork muscle tenderness and flavor by either grinding, mechanical tenderization, addition of water and cure ingredients, high humidity cooking, or any combination of these. These processes in turn reduce the variation in palatability normally seen in fresh pork and cause a tender product with distinctly different flavors. Additionally, of the 25% to 30% of the pork carcass that is marketed in a fresh state, approximately only one-half (12% to 15%) is sold through traditional retail meat channels to the consumer. The remainder is sold through food service channels in either a frozen state, or in a precooked and/or processed condition. These processes greatly reduce or eliminate normal variations in pork palatability.

In summary, although an increase in shear force in fresh pork was observed in some studies, as the result of RACT treatment, this effect is not of practical significance. Consumers should not observe small differences in sensory evaluation properties detected by trained sensory panelists in these studies. This series of performance, carcass and sensory trials confirms that RACT may be fed to pigs in a 16% crude protein diet to improve production efficiency and carcass composition without producing meaningful detrimental effects on the palatability of pork.

Table IV.6. Summary of Sensory Panel Evaluation and Warner-Bratzler Shear of Fresh Pork Loin Roasts for Combined 13 and 16 Percent Crude Protein Data[a]

	Treatment[b]							
	16-0	16-5	16-10	16-15	16-20	13-0	13-20	SE
Juiciness[c]	9.45	9.34	9.40	9.05	9.23	9.97	9.58	0.21

Tenderness[d]	10.20	9.86	10.13	9.77	9.72	10.52	10.04	0.20
Flavor[d]	9.96	9.75	9.74	9.89	9.93	10.08	9.83	0.11
Off Flavor[d]	14.97	14.98	14.99	14.99	14.99	14.99	14.99	0.007
WB Shear[d] (kg)	2.99	3.25	3.33	3.35	3.49	2.89	3.22	0.18

[a] Least squares means
[b]crude protein (%)-ractopamine (ppm)
[c]Weighted General Linear Model Analysis with weights being trial variances
[d]Mixed Model Analysis

Table IV.7. Summary of Sensory Panel Evaluation and Warner-Bratzler Shear of Cured Ham for Combined 13 and 16 Percent Crude Protein Data[a]

	Treatment[b]							
	16-0	16-5	16-10	16-15	16-20	13-0	13-20	SE
Juiciness[c]	9.00	9.16	8.90	8.85	8.96	8.92	8.92	0.20
Tenderness[d]	10.61	10.45	10.52	10.54	10.54	10.57	10.55	0.17
Flavor[d]	10.14	10.33	10.37	10.17	10.33	10.33	10.24	0.13
Off Flavor[e]	14.99	14.97	14.88	14.96	15.01	14.96	14.96	0.032
WB Shear[f] (kg)	1.68	1.68	1.67	1.68	1.66	1.62	1.69	0.06

[a]Least squares means
[b]crude protein (%)-ractopamine (ppm)
[c]Mixed model analysis - combined sexes
[d]Mixed model analysis
[e]Weighted General Linear Model Analysis with weights being trial variances
[f]Weighted General Linear Model Analysis with weights being trial variance - combined sexes

IV.D. Corroborative Dose Titration/Confirmation Clinical Effectiveness Trials For Performance Parameters

The objective of these trials was to evaluate the use RACT for improving average daily weight gain and feed efficiency in finishing swine when fed to an "equal weight" endpoint from approximately 150 to 240 lb including a 5-day pre-slaughter withdrawal. Animals from the trials were used as the pool of animals for selecting at least one and preferably two animals per pen for slaughter and determining changes in carcass leanness due to RACT as measured by percentage chemical lean.

Data were pooled from three replicated, well controlled trials involving 441 crossbred finishing pigs conducted in various swine production areas of the United States. Trial facilities were typical of those used by commercial swine operations in each location. Each trial contained pens of separately fed barrows and gilts. The trials were designed as "equal weight" trials in which the treatment period was terminated when each pen

weighed approximately 235 lb. At that time, the pen was placed on a 5-day pre-slaughter withdrawal period. At the end of the withdrawal period at least one and preferably two animals per pen weighing between 234 to 246 lb were selected for slaughter and determining carcass composition.

Each trial consisted of seven treatments:

1) a conventional 13% crude protein diet with no RACT (control)
2) a conventional 13% crude protein diet with 18 g/ton (20 ppm) RACT
3) a 16% crude protein diet with no RACT (control)
4) a 16% crude protein diet with 4.5 g/ton (5 ppm) RACT
5) a 16% crude protein diet with 9 g/ton (10 ppm) RACT
6) a 16% crude protein diet with 13 g/ton (15 ppm) RACT
7) a 16% crude protein diet with 18 g/ton (20 ppm) RACT

In each trial, seven treatment groups were replicated four times with two blocks of barrows and two blocks of gilts in a randomized complete block design. While seven treatments were used in the trials, only five treatments were used to determine the effective dose range of RACT for improvement in carcass leanness. Blocks were environmental locations within the finishing facilities. Within sexes, blocks consisted of contiguous pens. Initially, individual pigs weighed 150 lb ± 5.0% with the average pen weight being 150 lb ± 2.5%.

IV.D.1. Pooled Data from Three Corroborative Effectiveness Trials

Data from the three trials were pooled and analyzed. Variables of interest were average daily weight gain, average daily feed intake, feed to gain ratio and gain to feed ratio. Variables were statistically evaluated using either general linear models or mixed model methodology. Comparisons between controls and different levels of RACT in the 16% crude protein diets were made to evaluate the effectiveness for average daily weight gain and improved feed efficiency. Appropriate comparisons for the combined 13% and 16% crude protein data were made to evaluate certain safety aspects of the study.

The results of the statistical analysis of the 16% crude protein data are shown in Table IV.8. Pooled results for average daily weight gain showed that all non-zero levels of RACT in a 16% crude protein diet were numerically higher than controls. Average daily feed intake was reduced ($P \leq 0.05$) in pigs fed 16% crude protein containing 10, 15 and 20 ppm RACT compared to 16% crude protein controls. Feed efficiency (F/G) was improved ($P \leq 0.01$) for all non-zero levels of RACT in the 16% crude protein treatments compared to the 16% crude protein controls.

No adverse effects were observed for any treatments. In the 13% crude protein, 20 ppm treatment, the reduced response on growth performance may be partially attributed to insufficient levels of crude protein and hence amino acids (primarily available lysine). These data show that RACT fed in a 16% crude protein diet is effective for improving rate of body weight gain, feed efficiency and efficiency of gain when the product is withdrawn for 5 days before slaughter.

Table IV.8. Pooled Three Trial Summary of Performance Variables - Ractopamine Hydrochloride Corroborative Performance Trials

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)[b]	1.96	2.01	2.01	2.03	2.05	1.97	1.92
SE (lb/hd/day)[c]	0.096	0.096	0.096	0.096	0.096	0.096	0.096
Average Daily Feed Intake (lb)[b]	6.77	6.54	6.42	6.31	6.40	6.70	6.49
SE (lb/hd/day)[c]	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Feed to Gain Ratio[b]	3.48	3.28	3.20	3.11	3.13	3.40	3.39
Se[c]	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Gain to Feed Ratio[b]	0.289	0.306	0.313	0.322	0.320	0.294	0.296
Se[c]	0.006	0.006	0.006	0.006	0.006	0.006	0.006

[a]crude protein (%)-ractopamine (ppm)
[b]least square means
[c]standard error

IV.D.2. Individual Corroborative Effectiveness Trials:

Trial T4V319207**.** An "equal weight" dose titration trial was conducted in barrows and gilts fed separately by Austin J. Lewis, Ph.D. and Phillip S. Miller, University of Nebraska, Lincoln, NE. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 35 or 42 pigs per block and five or six pigs per pen. A total of 161 animals were used in the trial. Corn and soybean meal-based diets typical of the region were used in the trial. Feeding duration ranged from 40 to 52 days with feeding length consistent for pigs within a pen. Two animals were removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V319207

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	2.01	2.03	2.00	2.02	2.12	2.03	1.95
SD[b]	0.12	0.11	0.16	0.13	0.13	0.14	0.14
Average Daily Feed Intake (lb)	6.88	6.42	6.47	6.14	6.71	6.77	6.54
SD[b]	0.59	0.22	0.55	0.33	0.42	0.42	0.24
Feed to Gain Ratio	3.43	3.17	3.23	3.04	3.16	3.34	3.37
SD[b]	0.16	0.08	0.07	0.14	0.07	0.03	0.13
Gain to Feed Ratio	0.292	0.316	0.309	0.330	0.316	0.300	0.297
SD[b]	0.01	0.01	0.01	0.02	0.01	0.002	0.01

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

Trial T4V179208**.** An "equal weight" dose titration trial was conducted in barrows and gilts fed separately by Robert A. Easter, Ph.D. and Leroy Biehl, D.V.M., M.S., University of Illinois, Champaign-Urbana, IL. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 35 pigs per block and five pigs per pen. A total of 140 animals were used in the trial. Corn and soybean meal-based diets typical of the region were used in the trial. Feeding duration ranged from 33 to 52 days with feeding length consistent for pigs within a pen. Eight animals were removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V179208

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	2.14	2.25	2.23	2.14	2.23	2.12	2.06
SD[b]	0.07	0.06	0.27	0.15	0.17	0.19	0.14
Average Daily Feed Intake (lb)	7.10	7.01	6.85	6.56	6.82	6.97	6.84
SD[b]	0.38	0.35	0.86	0.50	0.33	0.52	0.54
Feed to Gain Ratio	3.32	3.12	3.08	3.06	3.07	3.29	3.33
SD[b]	0.20	0.16	0.15	0.16	0.20	0.06	0.07
Gain to Feed Ratio	0.302	0.321	0.326	0.327	0.327	0.304	0.301
SD[b]	0.02	0.02	0.02	0.02	0.02	0.01	0.01

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

Trial T4V319209**.** An "equal weight" dose titration trial was conducted in barrows and gilts fed separately by William Miller, Ph.D., Land O Lakes, Inc., Webster City, IA. Crossbred market hogs typical of industry practice were used in the trial. Two blocks of each sex were conducted with 35 pigs per block and five pigs per pen. A total of 140 animals were used in the trial. Corn and soybean meal-based diets typical of the region were used in the trial. Feeding duration ranged from 41 to 59 days with feeding length consistent for pigs within a pen. Two animals were removed during the trial for non-treatment related health reasons. No adverse reactions to RACT were observed.

Performance of Pigs Fed Ractopamine Hydrochloride - Trial T4V319209

	Treatment[a]						
Variable	16-0	16-5	16-10	16-15	16-20	13-0	13-20
Average Daily Weight Gain (lb)	1.72	1.74	1.81	1.92	1.80	1.77	1.76
SD[b]	0.14	0.10	0.11	0.09	0.15	0.17	0.10
Average Daily Feed Intake (lb)	6.32	6.17	5.93	6.23	5.66	6.36	6.09
SD[b]	0.33	0.36	0.10	0.43	0.58	0.66	0.46
Feed to Gain Ratio	3.69	3.54	3.29	3.24	3.14	3.58	3.47
SD[b]	0.21	0.18	0.14	0.17	0.09	0.14	0.17
Gain to Feed Ratio	0.272	0.283	0.304	0.309	0.318	0.279	0.289
SD[b]	0.02	0.01	0.01	0.02	0.01	0.01	0.01

[a]crude protein (%)-ractopamine (ppm)
[b]standard deviation

V. ANIMAL SAFETY:

V.A. Pivotal Studies

V.A.1. Target Animal Safety and Drug Tolerance Study (T4VVX8510)

A specifically designed Target Animal Safety and Drug Tolerance Study in crossbred swine was conducted to address the tolerance to and safety of feeding up to 500 ppm of RACT in the diet during a 56 day feeding period. Based on the results of this study, RACT is safe when administered in the diet at up to 20 ppm according to label directions.

a. Type of Study: This was a 56-day study in swine which were fed a complete diet containing 0, 20, 100 or 500 ppm RACT and observed for adverse effects.

b. Study Director: G. D. Williams, D.V.M., Ph.D. , Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN 46140

c. General Design:

1) Purpose: This study was designed to determine the toxicological effects of RACT administered in the diet to swine. Potential target organs and tissues were to be identified through clinical observations, gross necropsy and histological examination. Hematological parameters, body weight and feed consumption were other variables of interest.

2) Animals: Crossbred swine (4/sex/dose) were used in this study.

3) Control: Crossbred swine (4/sex) which received the same basal diet as the treated pigs, but without RACT.

4) Dosage form: A Type A Medicated Article containing 9 grams of RACT per pound was used to make a Type C Medicated Feed containing 20, 100, or 500 ppm of RACT. The Type A Medicated Article is the marketed form of Paylean®.

5) Dose: The diets which were fed *ad libitum* contained 0, 20, 100, or 500 ppm of RACT and based on actual feed consumption the resulting dosages (sexes combined) were 0, 0.6, 3, or 15 mg/kg body weight/day.

6) Route of Administration: Oral in the diet, provided *ad libitum*.

7) Study Duration: The live phase spanned 56 days of feeding medicated feed.

8) Pertinent measurements/observations: Daily clinical observations, mortality, hematological measurements, body weight, feed consumption, gross necropsy, and histopathology were evaluated to assess potential toxicity in swine.

d. Results:

Pigs tolerated dietary levels up to twenty-five times the highest intended use level of RACT without physical signs of toxicity. A mild decrease in erythrocyte number, hemoglobin concentration, packed cell volume and serum urea nitrogen concentration was observed in pigs fed 100 or 500 ppm RACT. Increase in liver periportal glycogen was also observed microscopically in a few pigs treated with 100 or 500 ppm. No toxicological effects were observed in pigs fed the highest intended commercial use level of 20 ppm RACT.

V.A.2. Reproductive Safety Study in Gilts (T4VVX8713)

A specifically designed Reproduction Performance Study in crossbred swine was conducted to identify the adverse effects, if any, of feeding RACT in the diet of gilts during a finishing period and subsequently diverting those gilts to the breeding herd.

Based on the results of this study, RACT is safe when administered in the diet at up to 20 ppm according to label directions.

a. Type of Study: Reproduction performance study in swine which were fed a complete diet containing 0, 20, or 60 ppm RACT during the finishing period (approximately 150 to 250 pounds or for 45 to 47 days) and observed for adverse effects on subsequent reproductive performance.

b. Study Director: G. D. Williams, D.V.M., Ph.D., Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN 46140

c. General Design:

1) Purpose: This study was designed to determine the reproductive performance effects of RACT administered in the diet to gilts during the finishing period and subsequently diverted to the breeding herd.

2) Animals: approximately 4 month old Hampshire x Yorkshire crossbred gilts, 44 per group

3) Control: approximately 4 month old Hampshire x Yorkshire crossbred gilts, 44 per group which received the same basal diet as the treated pigs, but without RACT.

4) Dosage form: A Type A Medicated Article containing 9 grams of RACT per pound was used to make a Type C Medicated Feed containing 20 or 60 ppm of RACT. The Type A Medicated Article is the marketed form of Paylean®.

5) Dose: The diets which were fed *ad libitum* contained 0, 20 or 60 ppm of RACT during the treatment period from an average of 147 lb body weight to an average of 245 lb of body weight.

6) Route of Administration: Oral in the diet, provided *ad libitum*.

7) Study Duration: The RACT feeding phase spanned 47 days for replicate one and 45 days for replicate two. After withdrawal of RACT from the diet, the gilts were bred, and allowed to farrow, and to nurse their litters to 21 days of age.

8) Pertinent measurements/observations: Daily clinical observations, mortality, hematological measurements, body weight, feed consumption, gross necropsy, and histopathology were evaluated to assess potential toxicity in swine.

d. Results:

Gilts fed either 20 ppm or 60 ppm RACT during the finishing period had improved average daily gain ($P<0.05$) and feed to gain ratio ($P<0.05$) when compared to control fed animals. There was no treatment-related effect ($P>0.10$) on any reproductive indices measured, including: percent of gilts observed in heat, farrowing rate, number of live or dead pigs farrowed, birth weight of live or dead pigs, number of pigs weaned at 21 days, weaning weights, total food consumed per gilt during lactation, or the weight of gilts at the end of lactation (21 days post-farrowing).

It was concluded that consumption of RACT in the diet during the finishing phase of gilt production would not adversely affect reproductive performance after the drug was withdrawn.

V.A.3. Change in the Ractopamine Hydrochloride Bulk Drug

Subsequent to the completion of the target animal safety studies, the process for manufacturing the bulk drug RACT was altered, resulting in a small shift in the enantiomer ratio of the four enantiomers comprising RACT. The shift was demonstrated to result in an about 15% increase in the RR isomer, which has been demonstrated to possess virtually all of the beta-agonist activity of the mixed enantiomer commercial product. The impact of this manufacturing alteration on the target animal safety studies was evaluated.

In the target animal safety and drug tolerance study, feeds containing 0, 20, 100, and 500 ppm of ractopamine hydrochloride were tested. Since these feed levels were based on the total enantiomer content of the older bulk drug, it was calculated that the levels tested were equivalent to 0, 17, 85, and 425 ppm of the new bulk drug, based on the increase in RR content (0.85 x 0, 20, 100, and 500 ppm respectively). Since no practically significant adverse effects were observed through the highest dose tested, it was concluded that the feed levels that had been tested were adequate to demonstrate the safety of RACT at up to 20 ppm in the feed of swine. This conclusion was further supported by the lack of any adverse effects from the feeding of the new bulk drug process RACT, at up to 20 ppm in the feed, in the pivotal and collaborative clinical effectiveness studies.

A similar evaluation of the results of the reproductive performance study resulted in a calculation of equivalent levels of 0, 17, and 51 ppm (0.85 x 0, 20, and 60 ppm respectively) being used based on RR enantiomer content of the new bulk drug. Because there were no adverse effects of RACT on reproductive performance at any feed level, it was concluded that the feed levels tested were sufficiently high to adequately demonstrate the safety of feeding up to 20 ppm of RACT with regard to impact on subsequent reproduction in gilts.

VI. HUMAN SAFETY:

VI.A. Toxicity Studies

VI.A.1. Subchronic and Chronic Studies.

VI.A.1.a. A Three-Month Subchronic Toxicity Evaluation of 031537 (Ractopamine Hydrochloride) Administered Orally to Fischer 344 Rats. Study R06184. December, 1984.

INVESTIGATOR: G. D. Williams, D.V.M., Ph.D.
Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

A three-month subchronic toxicity study was conducted in Fisher 344 rats (20/sex/dose) maintained on diets containing fixed levels of ractopamine hydrochloride: 0.0, 0.002; 0.02 and 0.2%. The time-weighted average daily dose of ractopamine hydrochloride was 0.0, 1.3, 13.4, or 152.9 mg/kg for males and 0.0, 1.4, 15.3, or 156.8 mg/kg for females.

All of the treated animals survived the treatment period. The observed effects were those expected with treatment with high levels of a sympathomimetic amine with thermogenic properties, i.e. decreased body weight gain, increased food consumption and decreased efficiency of food utilization. These signs were observed throughout the study only in the high-dose rats of both sexes. There were biologically significant changes in hematology, clinical chemistry and urinalysis parameters.

The only compound-related histopathologic change was a morphologic alteration of periaortic and interscapular brown fat, which was consistent with metabolic activation of brown fat. The morphologic alteration of brown fat was dose related with slight and moderate effects at the mid- and high-dose groups, respectively. No effect was seen at the lowest dose.

Treatment-related trends in organ weights of mid and high-dose group rats of both sexes were not considered to be of toxicological significance since they were not associated with functional or morphologic changes. These differences consisted of a slight decrease in liver and spleen weights and a slight increase in kidney weight. In female high-dose group rats, there was also a moderate decrease in the weight of the uterus.

No treatment-related effects were observed in the low-dose (0.002%) group rats of either sex (time-weighted average daily dose of 1.3 and 1.4 mg/kg for males and females, respectively). Therefore, the NOELs for this study were: 1.3 and 1.4 mg/kg body weight/day for males and females respectively.

VI.A.1.b. A Three-Month Toxicity Study of Ractopamine Hydrochloride Fed in the Diet to B6C3F1 Mice. Study M01584. June, 1987.

INVESTIGATOR: G. D. Williams, D.V.M., Ph.D.
Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

This three month subchronic toxicity study was conducted in B6C3F1 mice. Ten mice per sex per dose were maintained on diets containing fixed levels of 0.0, 0.02, 0.14, and 1.0% ractopamine hydrochloride, resulting in estimated time weighted average daily doses of 0.0, 25, 175, or 1250 mg/kg/day, respectively.

All of the treated animals survived the dietary exposure period. There were no treatment-related effects on body weight and no induction of the hepatic microsomal enzyme p-nitroanisole O-demethylase. Increases occurred in erythrocyte number, hemoglobin concentration, and packed cell volume in both sexes of the middle and high-dose groups. Urea nitrogen and cholesterol concentrations were increased in the high dose male mice and in both the middle and high-dose female mice. Treatment-related differences in weights of testes and heart were not accompanied by morphologic alterations. The dose-related decrease in the testes to body weight ratio occurred in all treatment groups of male mice. The effect in the low dose group was minimal. An increase in heart to body weight ratio occurred in the high dose mice of both sexes. Brown adipose tissue was darker in color in the high dose mice and, microscopically, the morphology was consistent with markedly increased metabolic activity. The middle dose female mice had brown fat of grossly normal color, but there was cytoplasmic change consistent with minimally increased metabolic activity.

The no effect level for females was the low dose of approximately 25 mg/kg. The only effect in males at the 25 mg/kg dose was a minor decrease in testicular weight. A NOEL was not established for males in this study.

VI.A.1.c. A Chronic Toxicity Study of Ractopamine Hydrochloride Administered Orally to Beagle Dogs for One Year. Study D05885. June, 1987.

INVESTIGATOR: G. D. Williams, D.V.M., Ph.D.
Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

This one year chronic toxicity study was conducted in beagle dogs (4/sex/dose) to provide data for the estimation of safety of this compound in a non-rodent species. The controls received capsules with marumerized vehicle and the treated dogs received capsules with 2% marumerized premix doses of 0.112, 0.224, and 5.68 mg/kg/day RACT for low-, middle-, and high-dose groups, respectively. The total daily dose was administered as three equally divided doses starting at approximately 7:00 a.m. with about six hours between each dose.

Body weight, food consumption, ophthalmic examinations, electrocardiogram waveforms, bone marrow evaluations, and urinalysis results were not affected by treatment. Treatment-related clinical observations were limited to oily, unkempt haircoats in high-dose dogs and to transient peripheral redness (abdominal skin, ears, and gums) primarily in high dose dogs. A few instances of transient

peripheral redness occurred early in middle dose dogs, but none was observed after the fifth month.

Slightly decreased erythrocyte number, hemoglobin concentration, and packed cell volume percent were present in the high dose dogs of both sexes throughout the study. Serum potassium and urea nitrogen concentrations were increased, and glucose, cholesterol, and triglyceride concentrations were decreased in the high dose group dogs throughout the study. Slightly decreased amounts of abdominal and/or thoracic fat, microscopic changes consistent with metabolic activation of the perithymic and/or periaortic brown fat, and depletion of hepatic glycogen were limited to the high dose dogs evaluated at the end of one year.

A dose related decrease in absolute heart weight was statistically significant for high dose dogs of both sexes and was associated with a mild but statistically significant decrease (26 beats/minute) in resting heart rate (bradycardia). The bradycardia seen at all RACT levels was most pronounced during the first six months and returned to near normal by the seventh month.

Treatment related effects on clinical observations, hematology and clinical chemistry parameters, organ weights, and gross and microscopic pathology were limited to the high dose (5.68 mg/kg/day) dogs. Except for the resting bradycardia, which returned to near normal in dogs in all dose groups during the last six months of the study, the no effect dose was the middle dose (0.224 mg/kg/day). A NOEL was not established for this study due to the resting bradycardia at all dose levels.

VI.A.1.d. A Subchronic Toxicity Study of Ractopamine Hydrochloride Given by Nasogastric Gavage to Rhesus Monkeys for 6 Weeks. Study P00691. October, 1993.

INVESTIGATOR: G. D. Williams, D.V.M., Ph.D.
and J. R. Shoufler
Toxicology Division
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Greenfield, IN 46140

This six week subchronic toxicity study was conducted in rhesus monkeys (2/sex/dose) to determine the dose at which toxic effects occurred so that appropriate effect and no-effect doses could be selected for a 1-year chronic study in monkeys. The compound was dissolved in purified water and given once daily by nasogastric gavage in a volume of 1 ml/kg. Control monkeys received vehicle (purified water) only. Treated monkeys received 0.25, 0.5, or 4.0 mg/kg of RACT for low-, middle-, and high-dose groups, respectively.

All monkeys survived the treatment period. Treatment had no effect on body weight, food consumption, ophthalmic examination, daily observations, electrocardiogram wave forms, or on hematology, clinical chemistry, or urinalysis

parameters. The physical examination at treatment termination was normal for all animals. There was no induction of the hepatic enzyme p-nitroanisole O-demethylase, no treatment-related changes in organ weights, and no gross or microscopic lesions.

The only significant effects of treatment were increased heart rate at the high dose of 4.0 mg/kg and a decrease in the number of beta adrenergic receptors in the lungs of monkeys from both the 0.5- and 4.0-mg/kg treatment groups. Heart rate in the 4.0-mg/kg treatment group monkeys was maximally increased during the first 4 hours after dosing. The mean heart rates after the first dose (0.5 through 4 hours) were 157, 148, 156, and 220 beats per minute for control, low-, middle-, and high-dose groups, respectively. The mean nighttime or resting heart rates (12 through 16 hours after the first dose) slowed to 107 beats per minute in the control but only to 134 beats per minute in the high-dose group monkeys. The high-dose group monkeys maintained a similarly elevated nighttime heart rate compared to controls throughout the remainder of the treatment period. The increased heart rates in control and all treatment group monkeys at 20 and 24 hours corresponded to turning on the lights in the animal rooms and were associated with their increased activity during the daytime hours.

A statistically significant decrease was observed in the number (Bmax) of lung beta-adrenergic receptors in the combined sexes of both the 0.5- and 4.0- mg/kg dose groups. In the 0.25-mg/kg dose group monkeys, Bmax was similar to the control group. There was no treatment effect at any of the doses tested on the affinity of the receptors for the radioligand, [3H]dihydroalprenolol.

In conclusion, monkeys given 4.0 mg RACT/kg body weight/day developed a daily heart rate increase and did not demonstrate a significant slowing of their nighttime heart rates. No evidence of myocardial damage was seen. A decrease in the number of the beta-adrenergic receptors in the lung occurred in both the 0.5- and 4.0-mg/kg treatment group monkeys. The no-observable-effect dose (NOEL) for this study was determined to be 0.25 mg/kg.

VI.A.1.e. A Chronic Study to Evaluate the Toxicity of Ractopamine HCl in a Nonrodent Species Whose Cardiovascular Responsiveness Approximates That of Humans Given This Class of Compounds. Study P05191. September, 1993.

INVESTIGATOR: G. D. Williams, D.V.M., Ph.D.
Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

This 1-year chronic toxicity study was conducted in rhesus monkeys for the purpose of helping to establish human food safety for potential residues of RACT in animals fed the drug. There were 4 monkeys/sex/treatment in this study except for the low-dose males. There were only three low-dose males due to an injury of

one male prior to the beginning of treatment. The animals were dosed once daily in the morning by nasogastric gavage. Controls received 1 ml/kg of purified water and treated animals received 0.125, 0.5, or 4.0 mg RACT/kg in an equivalent volume of purified water.

All monkeys survived the treatment period and no clinical signs attributable to treatment were observed. Food consumption, physical and ophthalmic examination, hematology, clinical chemistry, urinalysis, and gross and microscopic pathology were not affected by treatment. A significant increase in body weight occurred in the monkeys treated with 4.0 mg RACT/kg.

The most striking effect of treatment was cardiostimulation, an expected pharmacological effect of a beta-adrenergic agonist. The degree and extent of cardiostimulation occurred in a dose-related fashion at the 0.5- and 4.0-mg/kg doses. The increased heart rate was maximal during the first 4 hours after dosing. Resting or nighttime heart rates were also significantly increased compared to controls. There was no accommodation to the increased heart rates during the 1-year period of treatment. Heart weight relative to body weight was decreased in females for the mid- and high-dose groups and a similar trend for decreased heart weight was also observed in males of the corresponding dose groups. The number (Bmax) and affinity (Kd) of heart beta-adrenergic receptors for [3H]dihydroalprenolol was not affected. The only other treatment-related change was a decrease in the number of lung beta-adrenergic receptors in both sexes of the 4.0-mg RACT/kg treatment group.

RACT was rapidly absorbed after an oral dose. The time to a peak plasma concentration (Tmax) was approximately 1 hour after an oral dose of 4.0 mg/kg. Mean peak plasma concentrations were similar throughout the year (44 to 59 ng/ml) and were not different for males versus females. Plasma drug concentrations in monkeys treated with 0.125 and 0.5 mg RACT/kg were below the limit of detection (<5 ng/ml) for the assay.

In conclusion, treatment-related effects on heart weight and heart rate were observed in monkeys treated with 0.5 and 4.0 mg RACT/kg, and the increase in body weight and decrease in the number of lung beta-adrenergic receptors was present only in monkeys treated with 4.0 mg RACT/kg. The no-effect dose (NOEL) was the low dose of 0.125 mg RACT/kg.

VI.A.2. Special Cardiovascular Studies

VI.A.2.a. Special Study: The Hemodynamic Effects of Intravenous Administration of Ractopamine Hydrochloride in Anesthetized Dogs. Study DC1485. April, 1987.

INVESTIGATOR: D. N. Stone, Ph.D.
Toxicology Division
Lilly Research Laboratories

Division of Eli Lilly and Company
Greenfield, IN 46140

This study was conducted to evaluate the acute cardiovascular effects of ractopamine HCl. Anesthetized beagle dogs (two male, two female) were prepared for hemodynamic monitoring and the response to a 10-minute intravenous infusion of RACT at 35 µg/kg was determined.

Heart rate increased approximately 65% during the 10-minute infusion and remained elevated by at least 50% throughout the following 30-minute monitoring period. Mean arterial pressure fell sharply to approximately one half its control level during the infusion. Mean arterial pressure subsequently returned towards the control level, but remained depressed by approximately -25% at 40 minutes post infusion. The fall in mean arterial pressure was accompanied by an increase in pulse pressure as a result of the relatively greater reduction in diastolic as compared to systolic pressure.

Cardiac output increased approximately 50% during the infusion, and then stabilized at a level approximately 40% higher than control for the next 30 minutes. The increase in cardiac output was due primarily to an increase in heart rate, since stroke volume only transiently increased approximately 12% at the start of infusion and later decreased to approximately -18% of its control level. Total peripheral resistance fell sharply to approximately -65% of its control level immediately after the onset of infusion. Resistance subsequently returned to a level approximately one half of its control level for the next 30 minutes.

Peak aortic flow increased approximately 90% immediately after the onset of infusion and subsequently stabilized at approximately 70%. The aortic flow ejection period shortened by approximately -27% during the infusion and remained shorter for the next 30 minutes. Changes in the time to peak flow were small and variable.

The systemic cardiovascular effects included tachycardia and peripheral vasodilatation. Despite concomitant and potent augmentation of cardiac output, the magnitude of the peripheral vasodilatation was sufficient to cause a dramatic fall in mean arterial pressure.

Cardiac output increased as a result of the pronounced increase in heart rate, and in spite of the decrease in left ventricular stroke volume. The decrease in stroke volume was accompanied by other changes in the aortic flow waveform consistent with an increase in left ventricular contractility. These included an increase in peak aortic flow and a reduction in the duration of the ejection period.

VI.A.2.b. Special Study: The Hemodynamic Effects of Intravenous Administration of Ractopamine Hydrochloride in Anesthetized Monkeys. Study P07585. February, 1987.

INVESTIGATOR: D. N. Stone, Ph.D.

Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

This experiment was performed to determine the appropriateness of a primate model for safety assessment. Anesthetized Rhesus monkeys (2 male, 2 female, 2.5 - 3.5 kg body weight) were prepared for hemodynamic monitoring and the response to a 10-minute intravenous infusion of RACT at 35 µg/kg was determined.

All animals survived the experimental phase of this terminal study, and no signs of toxicity were observed.

Heart rate increased approximately 20% during the infusion and remained elevated throughout the monitoring period. Mean arterial pressure was maintained throughout the experiment while there was a slight augmentation of systolic pressure during the infusion. Cardiac output increased approximately 35% during the infusion and decreased steadily throughout the monitoring period. Total peripheral resistance decreased gradually to approximately -30% of its initial level during the infusion and then slowly returned towards control. Stroke volume increased approximately 14% during the infusion and exhibited a gradual return to baseline during the monitoring period. Peak aortic flow increased approximately 80% during the infusion and gradually returned toward baseline levels. The aortic flow ejection period shortened by approximately -18% during the infusion and remained so during the monitoring period. Changes in the time to peak flow were small and variable.

Changes in heart rate and arterial pressures observed under anesthetized conditions were qualitatively and quantitatively similar to those previously documented for this species. These included tachycardia and slightly increased pulse and mean arterial pressures. In the conscious state, the hemodynamic responses to ractopamine infusion were also tachycardia and slightly increased pulse and mean arterial pressures.

Barbiturate anesthesia in the Rhesus monkey does not alter the qualitative acute hemodynamic response to a 10-minute infusion of RACT at 35 µg/kg body weight.

VI.A.2.c. Special Study: The Effects of Intravenous Administration of Ractopamine Hydrochloride Compared in Conscious and Anesthetized Monkeys. Study P07685. June, 1987.

INVESTIGATOR: D. N. Stone, Ph.D.
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In the course of animal experimentation designed to assess the safety of the compound, species dependent differences in its cardiovascular effects have been demonstrated. Specifically, when infused as an intravenous solution at 0.035 mg/kg over a 10-minute period, RACT caused a profound fall in mean arterial pressure in pentobarbital anesthetized dogs, but not in pentobarbital anesthetized monkeys.

The present experiments were therefore performed to further characterize the primate response to infusion of RACT. Rhesus monkeys (8 males, approximately 6 kg body weight) were prepared for hemodynamic monitoring and the response to a 10 minute intravenous infusion of RACT at 0.035 mg/kg was determined in both the awake, and in the anesthetized states, as compared to an infusion of NaCl.

All animals survived the study, and no signs of toxicity were observed.

a. Arterial Pressure, Conscious State

Pulse pressure was increased due to the increase in systolic pressure, which appeared virtually immediately with the onset of infusion. Following the peak response approximately 8 minutes later, systolic pressure declined slowly to the control level over the next 90 minutes and was stable throughout the subsequent 6 hour monitoring period.

b. Arterial Pressure, Anesthetized State

Pulse pressure was increased by virtue of both an increase in systolic, and a decrease in diastolic pressure. The increase in systolic pressure appeared virtually immediately with the onset of infusion and continued to increase throughout the 40-minute monitoring period. The decrease in diastolic pressure was shorter and less pronounced than the increase in systolic pressure.

c. Heart Rate, Conscious State

Heart rate increased from its control level of approximately 120 beats per minute to a maximum of almost 190 beats per minute by the end of the 10-minute infusion. Thereafter, heart rate declined rapidly with the cessation of infusion.

d. Heart Rate, Anesthetized State

Heart rate increased to approximately 214 beats per minute by the end of the 10 minute infusion period. Thereafter, heart rate declined, yet it remained elevated for the duration of the 40-minute monitoring period.

Conclusion

The changes in heart rate and arterial pressures observed under anesthetized conditions were qualitatively and quantitatively similar to those previously documented for this species. These included tachycardia and slightly increased pulse and mean arterial pressure. In the conscious state, the hemodynamic responses to RACT infusion also resulted in tachycardia and slightly increased pulse and mean arterial pressures.

Barbiturate anesthesia in the Rhesus monkey does not alter the qualitative acute hemodynamic response to a 10-minute infusion of RACT at 35 µg/kg body weight.

VI.A.2.d. An Acute Cardiovascular Toxicity Study with Ractopamine HCl (Compound 031537) Administered Orally in the Conscious Instrumented Beagle. Study DV0193. September, 1993.

INVESTIGATOR: R.D. Sarazan D.V.M., Ph.D., B.W. Main, M.S.
Toxicology Research Laboratories
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The purpose of this study was to provide acute toxicity data on the cardiovascular effects of oral doses of RACT. Beagle dogs were orally administered 0, 2, 50, or 125 µg RACT/kg body weight. The low dose of 2 µg/kg was selected to be higher than the maximum human exposure anticipated from eating 125 grams of pig kidney from an animal that had not been removed from RACT treatment prior to slaughter. (At zero time withdrawal, pig kidney has the highest concentration of tissue residue at approximately 0.6 ppm which would result in an exposure of 1.25 µg RACT/kg body weight in a 60 kg person consuming 125 grams of kidney.) The mid dose of 50 µg/kg was selected because this dose had produced some clinical signs (skin erythema) without any statistically significant increase in heart rate in a previous study. The high dose of 125 µg/kg was selected since it was known to result in increased heart rate in dogs and corresponded to the NOEL previously established in primates.

Four male and 4 female beagle dogs 10 to 19 months of age (approximately 10 kg body weight) were utilized. The study was designed as a double Latin square that allowed testing for residual effects. Left ventricular inotropic state, systemic arterial pressure, heart rate, and electrocardiograms were recorded. Cardiovascular responses to the three doses of RACT and a control vehicle were evaluated.

Left ventricular pressure, aortic blood pressure, and electrocardiograms were recorded. The peak value of the first derivative of left ventricular pressure (dP/dtmax) was used as an index of left ventricular inotropic state. Systolic, diastolic, mean aortic, and aortic pulse pressures were derived by the data acquisition system from the aortic pressure signal. Heart rate and left ventricular end-diastolic pressure were obtained from the ventricular pressure signals.

All dogs survived the treatment. There was no residual carry-over effect from one treatment to the next in the Latin square design. RACT caused dose-dependent increases in heart rate and left ventricular inotropic state at the 50 and 125 µg/kg doses. No change in heart rate or left ventricular inotropic state was observed at the 2 µg/kg dose. Aortic pulse pressure decreased in response to treatment with the 50 and 125 µg/kg doses. A drop in blood pressure was evident in both the systolic and diastolic (and therefore the mean) pressures. The 125 µg/kg dose caused a decrease in aortic pulse pressure. Analysis of electrocardiograms did not indicate any treatment-related effects in the dogs. Two dogs in the 50 µg/kg dose group and seven dogs in the 125 µg/kg dose group demonstrated a slight pinking of the abdominal skin (erythema). The 2 µg/kg dose did not display any effect on any of the parameters measured in this study and was determined to be the NOEL.

VI.A.2.e. Special Study: The Effect of Subchronic Administration of Ractopamine Hydrochloride on Heart Rate and Electrocardiographic Waveforms in Conscious Rhesus Monkeys. Study P02186. May, 1987.

INVESTIGATOR: D. N. Stone, Ph.D. and
G. D. Williams, D.V.M., Ph.D.
Toxicology Division
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Division of Eli Lilly and Company
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This three month special cardiovascular study was conducted in rhesus monkeys (3/sex/dose) to provide supportive information for the evaluation of the safety of the compound. Two groups, each composed of three male and three female rhesus monkeys, were administered either vehicle or 0.125 mg RACT per kg body weight once per day for 90 days by nasogastric gavage. The test article was prepared as a solution in NANOpure water at a concentration of 0.125 mg/ml. Monkeys received 1.0 ml/kg of vehicle or the RACT solution and were maintained in a conscious state during the dosing and the cardiovascular monitoring procedures.

All of the monkeys were clinically normal throughout the study. There were no effects on body weight, food consumption, heart rate, or on the electrocardiographic waveforms in any control or treated monkeys.

In conclusion, it was established that rhesus monkeys given a daily oral dose of 0.125 mg RACT per kg body weight for 90 days had no evidence of compound

related changes in heart rate or electrocardiographic wave forms. The NOEL for this study is 0.125 mg RACT/kg body weight/day.

VI.A.2.f. Cardiovascular Activity and Safety of Ractopamine Hydrochloride: Determination of a No-effect Dose. Study T4V-LC-ERAA. July, 1994.

INVESTIGATOR: Thomas L. Hunt, MD., Ph.D.
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706 Banister Lane
Austin, Texas 78704

It was demonstrated in the one year chronic toxicity study in beagle dogs (study D05885, see VIA.7. above) that dogs were more sensitive to the cardiovascular effects of ractopamine that were rodents or primates. The purpose of this human study was to determine whether the dog or non-human primate provides a better model for estimating the acute toxicity of RACT in the human diet. The following study was conducted in humans under an IND established with the Center for Drug Evaluation and Research (CDER). The protocol for this study was jointly developed with the cooperation and concurrence of CDER, CVM, and Elanco Animal Health.

The dose-response effect of RACT on the human cardiovascular system was studied in a single-blind, placebo-controlled, ascending single-dose protocol. The study was conducted with six healthy male volunteers, given oral placebo plus five oral doses that ranged from 5 through 40 mg. Through standard and echocardiographic methods, measurements were obtained at 9 hourly time points in each subject for 14 cardiovascular variables. Characterization of the dose-response relationships provided calculated estimates of the NOEL. Based on a population modeling strategy, the following NOEL confidence intervals (95%) were calculated for three salient variables of cardiac function and for a composite over all variables:

Variable	NOEL (µg/kg)	Confidence Interval	
		Lower	Upper
Electromechanical systole	93	44	141
Heart Rate	116	62	169
Cardiac Output	83	35	132
Composite	99	92	106

The oral bioavailability of the RACT was estimated in this study by measuring the amount of free and conjugated ractopamine in the urine of the six subjects during the 24 hours following the oral dosing. Forty-five percent of the administered dose was collected in the urine in 24 hours. This compared favorably with the 24-hour excretion in dogs and monkeys of 54% and 42% respectively.

As a result of this study, it was concluded that the primate model is more predictive of the human acute toxicity response to oral exposure to RACT than the canine model.

VI.A.3. Reproduction and Developmental Studies.

VI.A.3.a. An Eleven Month Two Generation Reproduction Study, Including a Teratology Segment, in CD Rats Maintained on Diets Containing Ractopamine Hydrochloride. Studies R11385 and R18985. June, 1987.

INVESTIGATOR: J.A. Hoyt, MS, MA,
and G. D. Williams, D.V.M., Ph.D.
Toxicology Division
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Division of Eli Lilly and Company
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A two-generation reproduction study in CD rats was conducted to provide information concerning the effect of RACT on reproductive performance in rats. RACT was administered continuously, as a component of the diet, at levels of 0, 0.0002, 0.002, 0.02 and 0.2%. Time-weighted estimates of test article intake during the growth periods, derived by combining group values from both generations, were ca 0, 0.15, 1.4, 15 and 160 mg/kg/day for the male rats. Average prepartum test article intake for females closely paralleled the values for the males of the respective groups. Average test article intake by nursing dams was ca 2.6X the intake levels during late gestation.

In the F_0 generation, weanling male rats (25/group) were maintained on treatment diets for a 10-week growth period. Weanling female rats (25/group) were maintained on control diets for the first eight weeks and were given treatment diets during the ninth and tenth weeks of the growth period. Both sexes were continued on treatment diets throughout one breeding trial and until termination of the F_0 generation. The females were allowed to deliver and rear their f_{1a} progeny through postpartum day 21. Twenty-five f_{1a} weanling pups/sex/treatment group were selected to become F_1 parents and were maintained on the respective treatment diets. Following a 10-week growth period, the F_1 rats from corresponding treatment groups were mated. The females were allowed to deliver and rear their f_{2a} progeny through postpartum day 21. After a rest period, the F_1 animals from corresponding treatment groups were again mated. The females were killed on gestation day 20 for an assessment of intrauterine reproductive parameters and the collection of fetuses for external, visceral, and skeletal examinations. All F_0 and F_1 parental animals were necropsied and reproductive tissues were collected and prepared for histopathological evaluation.

Mortality was limited to the 0.2% group where two males and one female F_1 generation animals died during the sixth month on test. No gross lesions were found in the female and one male. The second male had severe progressive

glomerulonephrosis and thinness with marked depletion of abdominal, thoracic, and subcutaneous fat. No treatment related clinical signs of toxicity were evident.

Significant, treatment related depression in body weight and body weight gain occurred in both F_0 and F_1 males of the 0.2% group. Significant depression in body weight also occurred in F1 females of the 0.2% group. Food consumption was significantly increased during gestation in both the F_0 and F_1 females of the 0.2% group during the breeding trials for the f_{1a} and f_{2a} litters, respectively, and during the first week following parturition in F_0 females of the 0.002 and 0.02% groups. The transient increase in food consumption in the 0.002 and 0.02% groups was not considered to be of toxicological significance. During both the F0 and F_1 growth periods, efficiency of food utilization was significantly depressed in males of the 0.2% group. These findings with RACT were expected because weight loss and/or compensatory hyperphagia have been associated with a variety of sympathomimetic amines known to induce thermogenesis in animals.

Mating performance and fertility were not adversely affected by treatment with RACT. During the breeding trials for the f_{la} and f_{2a} litters, gestation length was not affected, but litter size, gestation survival, progeny survival, and progeny body weights were significantly depressed in the 0.2% group. On postpartum day 21, pups of the 0.2% group weighed approximately 20% less than those of the control group. Although the proportion of f_{1a} male pups was significantly depressed in the 0.2% group, there were no significant differences in sex distribution in the f_{2a} litters. Pallor, apparent hypothermia, thinness, dehydration and rough haircoat occurred with the highest frequency in neonatal and postnatal progeny of the 0.2% group. In addition, the incidence of abnormalities, which included edema, cleft palate, limb and shoulder anomalies, brachygnathia, protruding tongue, and open eyelid, were increased in the 0.2% group. These abnormalities were thought to have resulted from treatment related reduced uterine blood flow and/or increased maternal and fetal brown adipose tissue thermogenesis.

During the second breeding trial of the F_1 animals for the f_{2b} litters, numbers of corpora lutea and implantations were significantly depressed in the 0.2% group but the proportion of implanting ova was not significantly affected. However, the proportion of live fetuses was significantly depressed in the 0.2% group due to increases in both early and late resorptions. Fetal weights, the proportions of fetal runts, and sex ratios were not adversely affected. In the 0.2% group, there was a significant increase in the proportions of fetuses with developmental variations and abnormalities. Frequently observed abnormalities in the 0.2% group included edema, hydramnios, misshapen scapula, and limb anomalies. Frequently observed developmental variations in the 0.2% group included incomplete ossification of the calvaria, ribs, vertebral arches, ischium, and pubis; adrenal hemorrhaging; wavy ribs; and misalignment and incomplete fusion of sternal bars. These developmental alterations also were believed to be a consequence of treatment related reduced uterine blood flow and/or increased maternal and fetal brown adipose thermogenesis.

There were no treatment related histopathological findings in the F_0 or F_1 parental animals.

In conclusion, when two generations of male and female rats were maintained on diets containing 0, 0.0002, 0.002, 0.02, or 0.2% RACT, the 0.2% dietary level elicited toxicity. Parental toxicity was characterized by increased mortality, and developmental toxicity was characterized by increased mortality, structural abnormalities and growth retardation. Depressions in body weight, body weight gain and efficiency of food utilization at the 0.2% dietary level were expected from treatment with high levels of a thermogenic sympathomimetic amine. Mating performance and fertility were not adversely affected and developmental toxicity seen at the 0.2% dietary level was attributed to physiological changes that may be associated with the pharmacological action of RACT on blood flow and/or maternal and fetal brown adipose tissue thermogenesis. The NOEL for maternal and fetal effects was 0.02% of the diet, equivalent to approximately 15 mg/kg body weight/day of RACT.

VI.A.4. Genotoxicity Studies.

VI.A.4.a. The Effect of Ractopamine Hydrochloride on the Induction of Reverse Mutations in *Salmonella typhimurium* and *Escherichia coli* Using the Ames Test. Studies 951114AMS2000 and 951205AMS2000. January, 1996.

INVESTIGATORS: Michael L. Garriott, Ph.D. and Marcia A. Rexroat
Toxicology Research Laboratories
Lilly Research Laboratories
Division of Eli Lilly and Company
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The potential of RACT to induce bacterial mutation was evaluated in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and *Escherichia coli* strain WP2uvrA. Two tests were conducted with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor 1254-induced rats. Treatment with RACT did not result in the induction of *S. typhimurium* and *E. coli* revertants when tested at concentrations ranging from 312.5 to 5000 µg/plate in either the nonactivated or the activated assays.

VI.A.4.b. Ractopamine Hydrochloride Metaphase Chromosome Analysis of Human Lymphocytes Cultured In Vitro. Study DTA 5/941656. February, 1995.

INVESTIGATORS: Leslie C. Akhurst, B.Sc. (Hons.), C.Biol., M.I.Biol.
Huntington Research Centre, Ltd.
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This test was to assess the ability of RACT to induce chromosomal aberrations in human lymphocytes cultured in vitro. Cultured human lymphocytes were

stimulated to divide by addition of phytohaemagglutinin and exposed to RACT in the presence and absence of S9 mix derived from rat livers for the subsequent conduct of two tests.

In the first test, the following concentrations of RACT were tested in an 18-hour harvest: 50, 156.3, and 250 µg/ml without S9 mix; 300, 600, and 1000 µg/ml with S9 mix. In the second test, the following concentrations of RACT were tested in an 18-hour harvest: 25, 100, and 200 µg/ml without S9 mix; 75, 150, and 300 µg/ml with S9 mix.

In the first test, in the absence of S9 mix, no statistically significant increases in the proportion of metaphase figures with chromosomal aberrations occurred. In the second test, statistically significant increases in the proportion of aberrant metaphase figures occurred at the highest (200 µg/ml) and intermediate (100 µg/ml) concentrations. In both the first and second test in the presence of S9 mix, statistically significant increases in aberrant cells occurred at the highest dose levels analyzed (1000 and 300 µg/ml, respectively). These increases in both the absence and presence of S9 mix fall outside the historical control range of the performing laboratory and are indicative of clastogenic activity.

VI.A.4.c. Mutagenicity Test On Ractopamine Hydrochloride Measuring Chromosomal Aberrations in Human Whole Blood Lymphocytes With and Without Exogenous Metabolic Activation. Study 17282-0-449EC. September, 1996.

INVESTIGATORS: Hemalatha Murli, Ph.D.
Corning Hazleton Inc. (CHV)
9200 Leesburg Pike
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In a confirming study RACT, was evaluated for the ability to induce chromosomal aberrations in vitro in human lymphocytes. Cultures dosed with 150, 200, 250, and 300 µg/ml for 17.8 hours without metabolic activation and with 1200, 1500, 1990, and 2490 µg/ml for 3 hours with metabolic activation were evaluated for chromosomal aberrations. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed in the 17.8 hour nonactivated assay. A significant increase in cells with chromosomal aberrations was observed in the cultures dosed with the toxic dose of 2490 µg/ml in the 3 hour assay with metabolic activation. No significant increase in polyploidy or endoreduplication was observed at the concentrations analyzed in the activation assay. No reduction was observed in the mitotic index of the cultures dosed with 1200 µg/ml in the nonactivation aberration assay with a 3 hour treatment.

The nonactivation assay with a 3-hour treatment was repeated testing concentrations from 1000 to 2000 µg/ml, and cultures dosed with 1000 and 1100 µg/ml were evaluated for chromosomal aberrations. A weakly significant increase in cells with chromosomal aberrations was observed in the cultures dosed with 1100 µg/ml. No significant increase in polyploidy or endoreduplication was

observed at the concentrations analyzed. This assay was repeated and cultures dosed with 700, 800, 900, and 1000 µg/ml were evaluated for chromosomal aberrations. A significant increase in cells with chromosomal aberrations was observed in the cultures dosed with 800, 900, and 1000 µg/ml. No significant increase in polyploidy or endoreduplication was observed at the concentrations analyzed.

Based on these findings, RACT was considered negative for inducing chromosomal aberrations in cultured whole blood human lymphocytes without metabolic activation following an approximately 18 hour treatment, positive without metabolic activation after a 3 hour treatment at higher doses, and positive at a single toxic dose level with metabolic activation. RACT was considered negative for inducing polyploidy and end or eduplication in the human peripheral blood lymphocytes with and without metabolic activation.

VI.A.4.d. The Effect of Ractopamine Hydrochloride on the In Vitro Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells. Studies 951108CAB2000 and 951206CAB2000. February 1996

INVESTIGATORS: Maria S. Jackson and Michael L. Garriott, Ph.D.
Toxicology Research Laboratories
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The potential of RACT to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells was investigated in the in vitro chromosome aberration assay. The test was conducted both with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor 1254-induced rats. Chromosomal aberrations were evaluated in CHO cells exposed for 4 hours to RACT concentrations of 400, 800, 900, or 1000 µg/ml without metabolic activation, 850, 875, or 900 µg/ml with metabolic activation, or for 19 hours at concentrations of 65, 100, or 250 µg/ml without metabolic activation.

Treatment with RACT did not produce a statistically significant increase in chromosomal aberrations either with or without metabolic activation. However, treatment with RACT did produce a concentration-dependent increase in the percentage of cells with diplochromosomes in the absence of S9 mix at the three highest concentrations in the 4 hour treatment group. This indicated that the test article caused an increase in the incidence of endoreduplication. However, there is no known correlation between endoreduplication and chromosomal damage. It was concluded that RACT did not induce structural chromosomal aberrations in vitro in CHO cells.

VI.A.4.e. Mutagenicity Test On Ractopamine Hydrochloride (compound 031537) Measuring Chromosomal Aberrations In Vivo In Mouse Bone Marrow Cells. Study 17282-0-451. March 1996.

INVESTIGATORS: Hemalatha Murli, Ph.D.
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Vienna, VA 22182

The potential of RACT to induce chromosomal aberrations in the bone marrow of ICR (Hsd:(ICR)) mice was evaluated in this in vivo assay. An acute (one-time only) dosing regimen was used to administer the test article, which was solubilized in 10% gum acacia, by gavage.

Following a dose selection study, doses of 300, 600, and 1200 mg/kg of body weight were tested. Animals (5/sex/dose/harvest time) dosed with the test article and the vehicle control article were euthanized approximately 6, 18, and 30 hours after dosing for extraction of the bone marrow. Positive control (cyclophosphamide) groups euthanized approximately 18 hours after dosing and vehicle control groups euthanized approximately 30 hours after dosing were included in the assay. No significant increases in chromosomal aberrations were observed in any of the RACT dose groups from any of the harvest times as compared with the vehicle control groups.

VI.A.4.f. Mutagenicity Test On Ractopamine Hydrochloride (compound 031537) Measuring Chromosomal Aberrations In Vivo In Mouse Bone Marrow Cells. Study 17282-2-451. September, 1996.

INVESTIGATORS: Hemalatha Murli, Ph.D.
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Vienna, VA 22182

The potential of RACT to induce chromosomal aberrations in the bone marrow of ICR (Hsd:(ICR)) mice was evaluated in this in vivo assay following a subchronic (14 consecutive days) dosing regimen. RACT was solubilized in 10% gum acacia and administered by gavage.

Dose levels of 200, 400, and 800 mg/kg of body weight were tested. Animals (5/sex/dose) dosed with the test article and the vehicle control article for 14 consecutive days were euthanized approximately 24 hours after the last dose for extraction of the bone marrow. Positive control (cyclophosphamide) groups were euthanized approximately 24 hours after an acute single dosing. The animals were observed immediately after the first dosing and periodically throughout the duration of the study for toxic signs and/or mortality. Signs of toxicity were observed in all dose groups. Two males died in the 800 mg/kg dose group and one male died in the 400 mg/kg dose group. Four of five females died in the 800 mg/kg dose group and the remaining animal was not evaluated cytogenetically.

No significant increases in chromosomal aberrations were observed in any of the RACT dose groups as compared with the vehicle control group.

VI.A.4.g. The Effect of Ractopamine Hydrochloride Given Orally for 2 Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice. Study 930811MNT2000. December, 1993.

INVESTIGATORS: Michael L. Garriott, Ph.D., Linda S. Schwier, Joseph W. Parton, Gail D. Williams, D.V.M., Ph.D.
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Lilly Research Laboratories
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The potential of RACT to induce micronuclei in vivo was investigated in bone marrow of male and female ICR mice. Mice (5/sex/dose) were treated orally for 2 consecutive days with either 20 ml/kg of the vehicle (10% acacia), 185.75, 371.5, or 743 mg/kg of RACT, or 25 mg/kg of the promutagen cyclophosphamide, which was the positive control for this study. Approximately 24 hours after the second treatment with RACT, bone marrow was collected, and the frequency of micronucleated polychromatic erythrocytes (MPCE) was determined microscopically. The incidence of MPCE in mice treated with RACT was not different from that of animals receiving the vehicle control. The response to the positive control demonstrated that the test system was sensitive to a chemical clastogen. It was concluded that RACT did not induce micronuclei in vivo in bone marrow of ICR mice.

VI.A.4.h. The Effect of Ractopamine Hydrochloride Given Orally by Gavage for 14 Consecutive Days On The Induction of Micronuclei in Bone Marrow of Fischer 344 Rats. Studies 960610MNT2000 and 960815MNT2000. October, 1996.

INVESTIGATORS: Michael L. Garriott, Ph.D., Linda S. Schwier, and Joseph W. Parton
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The potential of RACT to induce micronuclei (MN) in vivo was investigated in bone marrow of male and female Fischer 344 rats. In the first study, rats (5/sex/dose) were treated orally by gavage for 14 consecutive days with either 20 ml/kg of the vehicle (10% aqueous acacia) or 50, 100, or 200 mg/kg of RACT. In the second study, rats (5/sex/dose) were treated with either 20 ml/kg of the same vehicle or 200, 400, or 600 mg/kg of RACT. The promutagen, cyclophosphamide, served as the positive control for both studies and was given at a dose of 5 mg/kg. Bone marrow was collected approximately 24 hours after the last treatment, and the frequency of micronucleated polychromatic erythrocytes (MPCE) was determined microscopically for each study.

There were no deaths in the first study, and all animals appeared normal throughout the test. Ractopamine induced statistically significant increases in MPCE in the bone marrow of male and female Fisher 344 rats under the treatment conditions.
In the second study, which was conducted at higher doses, two deaths occurred (one male in the mid low dose and one male in the high mid-dose groups) on Day 12. Clinical observations of low carriage, sternal recumbence, rapid breathing, hypoactivity, salivation, and lacrimation were observed beginning on Day 0 in all RACT treatment groups. The incidence of MPCE in male and female rats treated with RACT was not increased over animals receiving the vehicle control.

In conclusion, RACT induced statistically significant increases in MPCE in the bone marrow of both male and female Fisher 344 rats at doses of 50-200 mg/kg given by gavage for 14 consecutive days.

VI.A.4.i. Radiocarbon Concentrations in Bone Marrow and Plasma of Male ICR Mice after a Single Oral 748 mg/kg Dose of 14C-Ractopamine Hydrochloride (Compound 031537). Study M05895.

VI.A.4.j. Radiocarbon Concentrations in Bone Marrow and Plasma of Male Fischer 344 Rats after a Single Oral 200 mg/kg Dose (Free Base) of 14C-Ractopamine Hydrochloride. Study R13696.

INVESTIGATORS: Sylvia H. Chay, MS and Raymond C. Pohland, Ph.D.
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These two studies in mice and rats were conducted to provide evidence of exposure to RACT in the in vivo studies using mouse and rat bone marrow cells. The results of the studies (4 time points from 0.5 to 6 hours after treatment) confirm that the bone marrow compartment of mice and rats (4 animals/time point) were exposed to ractopamine and/or its metabolites under the conditions used in the experiments.

VI.A.4.k. The Effect of Ractopamine Hydrochloride On The Induction of Forward Mutation At The Thymidine Kinase Locus OF L5178Y Mouse Lymphoma Cells. Studies 951115MLA2000 and 951219MLA2000. October, 1996.

INVESTIGATORS: Michael L. Garriott, Ph.D. and David J. Yount
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The potential of RACT to induce mammalian cell mutations was investigated both with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor 1254-treated rats. A reproducible mutagenic response was observed in the nonactivated test with RACT at concentrations of 300, 325, and 350 µg/ml. A mutagenic response was also noted in the activated test at the concentrations of 200, 225, 250, and 275 µg/ml. It was concluded that RACT was mutagenic in L5178Y mouse lymphoma cell with and without metabolic activation.

VI.A.4.l. Mutagenicity Test On Ractopamine Hydrochloride In The L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay. Study 17282-0-431. September, 1996.

INVESTIGATORS: Maria A. Cifone, Ph.D.
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In a confirming study, the potential of RACT to induce forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line was evaluated. In the nonactivation trial, the test article was analyzed for mutant induction from 50 µg/ml to 225 µg/ml, and none of the six analyzed treatment concentrations induced a mutant frequency that exceeded the minimum criterion for a positive response. It should be noted that the apparent conflicting results

reported here and those reported under VI.A.4k above, in the absence of metabolic activation, may be related to the differences in the Ractopamine dose levels employed in the assay. In the presence of metabolic activation, treatments from 75 g/ml to 275 g/ml were analyzed. All five analyzable doses in the presence of metabolic activation system, induced mutant frequencies that were 2- to 3.4-fold above the concurrent vehicle control values and the increases in the mutant frequencies were dose-related.

VI.A.4.m. Analysis of Mouse Lymphoma Cell Culture Media for Ractopamine and Ractopamine Catechol using LC/MS/MS. November, 1996.

INVESTIGATORS: Anthony T. Murphy and Todd A. Gillespie, Ph.D.
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This non-GLP study was conducted to partially test the hypothesis that RACT induces a clastogenic effect through a secondary mechanism in vitro after undergoing an oxidation in the cell culture media. Mouse lymphoma cell cultures were prepared according to standard operating procedures for the assay.

The mouse lymphoma cell cultures were treated with either solvent control [1.0% dimethyl sulfoxide (DMSO)], 350 µg/ml RACT (RAC), 1000 µg/ml N-acetylcysteine (NAC) or a combination of 435 µg/ml S-adenosyl-L-methionine (SAM) plus 80 units catechol-O-methyl transferase (COMT). Other treatment combinations included: 350 µg/ml ractopamine plus 1000 µg/ml NAC; 350 µg/ml ractopamine plus 435 µg/ml SAM plus 80 units COMT; and 350 µg/ml ractopamine plus 1000 µg/ml NAC plus 435 µg/ml SAM plus 80 units COMT. SAM and COMT were added to the samples to methylate potentially unstable catechols and thus facilitate their detection.

All of the mouse lymphoma treatment combinations listed above were examined with and without the addition of rat hepatic S9 fraction. The treated mouse lymphoma cultures were analyzed using product ion scans and MRM (multiple reaction monitoring). Product ion scans were used to provide mass spectral confirmation of the ractopamine catechol and MRM was used to provide quantitative determination of the ractopamine catechol.

The presence of the ractopamine and ractopamine-catechol were confirmed using Product Ion scans. Product Ion scans of synthetic ractopamine and ractopamine-catechol were matched to Product Ion scans of media samples.

Accumulation of Ractopamine-catechol (RACT-catechol) occurred only in the presence of metabolic activation when the mouse lymphoma cells are treated with NAC, and in the absence of metabolic activation. The minor amounts of RACT-catechol observed under the other test conditions appear to be insignificant with respect to any catechol-mediated genotoxicity for ML cells.

VI.A.4.n. Effect of Ractopamine Hydrochloride on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells: Special Studies. (Studies 960313MLA2000, 960409MLA2000, 960416MLA2000, 960423MLA2000, 960723MLA2000. 960730MLA2000, 961015MLA2000, 961030MLA2000). November, 1996.

INVESTIGATORS: Thomal J. Oberly, MS and David J. Yount
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In this non-GLP mechanistic study, the potential of various chemical agents to eliminate, reduce, or enhance the genotoxic effect of RACT in the L5178Y TK+/- mouse lymphoma cell assay (MLA) was investigated. Ascorbic acid, propranolol, superoxide dismutase/catalase, copper (II) 3,5-diisopropylsalicylate hydrate, N-acetyl-L-cysteine, and N,N'-diphenyl-1,4-phenylenediamine were tested for the ability to block the mutagenic effect of RACT. These studies were performed without metabolic activation except superoxide dismutase/catalase was tested with and without metabolic activation. In addition, L-buthionine-[S,R]-sulfoximine, an inhibitor of GSH production (reduced glutathione), was evaluated for the ability to enhance the mutagenic effect of RACT and was tested without metabolic activation. Ethylmethanesulfonate (EMS) and 3-methylcholanthrene (3MC) were also tested and served as positive controls for the non-activated and activated assays, respectively. For the tests with metabolic activation, an S9 fraction prepared from the livers of Aroclor 1254-treated rats was used.

Similar studies were performed to examine the effect of various chemical agents on epinephrine-induced mutagenicity. Ascorbic acid, superoxide dismutase, copper (II) 3,5-diisopropylsalicylate hydrate, N-acetyl-L-cysteine, and N,N'-diphenyl-1,4-phenylenediamine were tested for the ability to modulate genotoxicity. These studies were performed without metabolic activation. The results of the epinephrine studies were compared to the results with RACT in the mouse lymphoma assay.

Treatment with either EMS or 3MC resulted in the induction of L5178Y TK-/- mutants and confirmed the sensitivity of the test system. The positive controls generally were not affected by the presence of the blocking agents. The following agents did not alter the mutagenicity of RACT in the MLA: the anti-oxidant ascorbic acid, superoxide dismutase (SOD), the β-blocking agent propranolol. The results failed to reveal any significant effects of the presence of catalase in reducing the mutagenic activity of Ractopamine in the MLA in the presence of metabolic activation. N-acetyl-L-cysteine and catalase reduced the mutagenic effect of RACT and N-acetyl-L-cysteine and ascorbic acid reduced the mutagenic effect of epinephrine in the mouse lymphoma assay.

The proposed mechanism of epinephrine-induced mutagenesis is based on oxidative injury due to the auto-oxidation of epinephrine from a catechol to a quinone. Whether this effect can mechanistically define the genotoxic response of ML cells to RACT remains to be further elucidated.

V1.A.5. Carcinogenicity Studies.

V1.A.5.a An Oncogenic Study in Fischer 344 Rats Given Ractopamine Hydrochloride in the Diet for 2 Years.

INVESTIGATOR: G. D. Williams, D.V.M., Ph.D.
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The oncogenic potential of RACT was assessed in Fischer 344 rats (60/sex/dose) administered the compound in the diet for 24 months. RACT doses were approximately 0, 2, 60, or 200 mg/kg/day for males and 0, 2, 60, 200, or 400 mg/kg/day for females. Plasma samples from rats at approximately 6, 12, and 18 months were analyzed for parent and total (parent + metabolite) RACT. Systemic exposure for both males and females at all doses was confirmed, and mean parent RACT concentrations ranged up to 24.4 ng/ml for the 200 mg/kg males and 31.1 ng/ml for the 400 mg/kg females. Total RACT concentrations ranged up to 19060 ng/ml for the 400 mg/kg females.

Survival was significantly increased for the 200 mg/kg males and 400 mg/kg females associated with decreased mean body weight and decreased mortality from chronic progressive nephropathy and common neoplasms. Decreased body weight coupled with increased food consumption in these groups was characteristic of β-adrenergic agonist mediated increased thermogenesis. The only neoplasm with increased incidence was the pharmacologically mediated costo-uterine leiomyoma in 6 and 27 of 60 female rats in the 200 and 400 mg/kg groups, respectively.

All other treatment-related findings in this oncogenic study in rats were also directly attributed to the to the β-adrenergic agonist pharmacologic activity of RACT.

The leiomyoma is a benign tumor in animals and humans. Studies on related adrenergic β-receptor agonists have shown this response to be blocked by the co-administration of an adrenergic β-receptor antagonists.[1,2,3] Therefore the

[1] Jack, D, D Poynter, and NW Spurling. 1983. Beta-adrenoceptor stimulants and mesovarian leiomyomas in the rat. *Toxicology* 27:315-320.
[2] Gibson, JP, DM Sells, HC Cheng, and L Yuh. 1987. Induction of uterine leiomyomas in mice by medroxalol and prevention by propranolol. *Toxicol. Pathol.*15(4):468-473.
[3] Gopinath, C and WA Gibson. 1987. Mesovarian leiomyomas in the rat. *Environ. Health Persp.* 73:107-113.

leiomyoma is considered to be a non-carcinogenic threshholdable event, similar to other toxicological endpoints.

V1.A.5.b Oncogenic Study and Companion Blood Level Study in CD-1 Mice Given Ractopamine Hydrochloride in the Diet for 21 Months.

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The oncogenic potential of RACT was assessed in CD-1 mice (60/sex/dose) administered the compound in the diet for 21 months. Mice were exposed to dietary RACT concentrations of 0,0.02%, 0.1% or 0.6% which provided approximately 0, 25, 130, or 840 mg/kg/day for males and 0, 35, 175, or 1085 mg/kg/day for females. Plasma samples from mice at approximately 6, 12, and 18 months were analyzed for parent and total (parent plus metabolites) RACT. Systemic exposure for both males and females at all doses was confirmed and mean parent and total RACT concentrations ranged up to 27.1 ng/ml and 17,840 ng/ml, respectively for the 0.6% group, (sexes combined).

The dietary concentration of 0.6% RACT exceeded the maximum tolerated dose for both males and females; increased mortality in this dose group was attributable to an enhanced severity of cardiomyopathy. Decreased mean body weight, coupled with increased food consumption in the 0.6% group was also observed. A treatment related production of uterine leiomyomas in female mice was observed at all treatment levels. The incidences of uterine leiomyomas in the 0, 0.02, 0.1 and 0.6% dose groups for female rats were 1.7, 8.3, 13.3 and 16.9%, respectively.. . All other treatment-related findings in this oncogenic study in mice were also directly attributed to the to the β-adrenergic agonist pharmacologic activity of RACT.

The leiomyoma is a benign tumor in animals and humans. Studies on related adrenergic β-receptor agonists have shown this response to be blocked by the co-administration of an adrenergic β-receptor antagonists.[4,5,6] Therefore the leiomyoma is considered to be a non-carcinogenic threshholdable event, similar to other toxicological endpoints.

Since uterine leiomyomas produced in the CD-1 mice was observed at all treatment levels, a NOEL could not be established for this tumorigenic endpoint.

[4] Jack, D, D Poynter, and NW Spurling. 1983. Beta-adrenoceptor stimulants and mesovarian leiomyomas in the rat. *Toxicology* 27:315-320.
[5] Gibson, JP, DM Sells, HC Cheng, and L Yuh. 1987. Induction of uterine leiomyomas in mice by medroxalol and prevention by propranolol. *Toxicol. Pathol.*15(4):468-473.
[6] Gopinath, C and WA Gibson. 1987. Mesovarian leiomyomas in the rat. *Environ. Health Persp.* 73:107-113.

A benchmark dose calculation was used to calculate a no effect level dose for this endpoint. The incidence rate for leiomyomas was adjusted for animal survival. A benchmark dose of 320 mg/kg/day was calculated based on an excess leiomyoma incidence of 10% above control group incidence, and using a lower 95% confidence limit. The 320 mg/kg/day benchmark dose is considered to correspond to a NOEL.

VI.B. Safe Concentration of Total Residues

VI.B.1. No-Observed-Effect Level (NOEL):

The safe concentration of total residue was determined from the lowest NOEL in the most appropriate species from the various toxicology studies conducted. A summary of the studies useful for determination of the Acceptable Daily Intake (ADI) follows:

Table 6.1. Summary of ADI Toxicology Studies for ractopamine hydrochloride

Study Type	Species	Doses mg/kg/day*	NOEL mg/kg/day*
90-day oral	Mouse	0, 25, 175, 1250	25
90-day oral	Rat	0, 1.3, 14.3, 154.8	1.3
Two-generation Reproduction	Rat	0, 0.15, 1.4, 15, 160	15
14-day oral	Dog	0, 0.05, 0.15, 1.5	0.05
1-year oral	Dog	0, 0.112, 0.224, 5.68	NA
90-day gavage	Monkey	0, 0.125	0.125
6-week gavage	Monkey	0, 0.25, 0.5, 4.0	0.25
1-year oral	Monkey	0, 0.125, 0.5, 4.0	0.125
2-year oral oncogenicity	Mice	0, 35, 175, 1085**	320***
Single dose oral	Man	5 - 40 mg total dose	0.1

*Doses based on ractopamine hydrochloride bulk drug.

**Female mice only.

***The oncogenicity study in mice resulted in the calculation of a benchmark dose rather than a NOEL. The benchmark dose calculated from the 90% lower confidence bound is still an effect level, and an additional 3-fold safety factor was applied to the 100-fold safety factor traditionally used for chronic study endpoints. Given benchmark dose value of 320 mg/kg/day, and a 300-fold safety factor, a reference dose (RfD, equivalent to an ADI) of 1 mg/kg/day was calculated. This value was well above the ADI calculated below for the 1-year oral toxicity study in the monkey.

The 1-year oral study in the monkey was selected as the study with the lowest appropriate NOEL for determining the acceptable daily intake (ADI). The NOEL was 0.125 mg/kg body weight of RACT bulk drug/day.

VI.B.2. Calculation of the Acceptable Daily Intake (ADI) and the Safe Concentration (SC) for ractopamine hydrochloride:

a. Acceptable Daily Intake (ADI)

$$ADI = \frac{NOEL}{\text{safety factor}} = \frac{0.125 \text{ mg/kg/day}}{100} = 0.00125 \text{ mg/kg/day} = 1.25 \text{ µg/kg/day}$$

b. Safe Concentration (SC)

$$(SC) = \frac{\text{Acceptable Daily Intake (ADI) x Human Weight}}{\text{Food Factor}}$$

Where: Human Weight = 60 kg

Food Factor: muscle = 300 g
fat = 50 g
liver = 100g
kidney = 50 g

$$SC \text{ (muscle)} = \frac{1.25 \text{ µg/kg/day x 60 kg}}{300 \text{ g/day}} = 0.25 \text{ µg/g} = 0.25 \text{ ppm}$$

$$SC \text{ (fat)} = \frac{1.25 \text{ µg/kg/day x 60 kg}}{50 \text{ g/day}} = 1.5 \text{ µg/g} = 1.5 \text{ ppm}$$

$$SC \text{ (kidney)} = \frac{1.25 \text{ µg/kg/day x 60 kg}}{50 \text{ g/day}} = 1.5 \text{ µg/g} = 1.5 \text{ ppm}$$

$$SC \text{ (liver)} = \frac{1.25 \text{ µg/kg/day x 60 kg}}{100 \text{ g/day}} = 0.75 \text{ µg/g} = 0.75 \text{ ppm}$$

Therefore, the safe concentration as total ractopamine hydrochloride (ppm) in edible swine tissue is:

Tissue	Calculated Safe Concentration (ppm)
Muscle	0.25
Fat	1.5
Kidney	1.5
Liver	0.75

VI.C. Total Residue Depletion and Metabolism Studies

VI.C.1. Characterization of ^{14}C- Residues in Tissues and Excreta from Swine Fed ^{14}C-Ractopamine HCl. Study ABC-0355.

INVESTIGATORS: J. E. Dalidowicz, Ph.D., and G.E. Babbitt, MS
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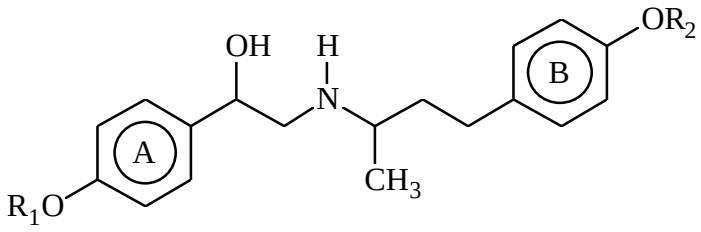
The purpose of this study was to characterize ^{14}C- residues in swine fed ^{14}C -RACT. Tissues from studies ABC-0273 and ABC-0291 and urine and tissues from studies ABC-0283 and ABC-0330 were used to identify and characterize the 14C -RACT residues.

In swine fed 30 ppm of ^{14}C-RACT in the diet, of the extractable liver residues, 30-50% was found to be parent RACT and the rest conjugates of RACT. The kidneys contained higher amounts of conjugates (64%) with a concomitant decrease in the amount of parent RACT (20-30%). Reverse-phase HPLC of the aqueous fraction containing the conjugates from both the liver and kidneys showed that it contained three metabolites of RACT (Metabolites A, B, C):



	R_1	R_2	Isomers
Ractopamine	H	H	Mixture
Metabolite A	H	Glucuronide	RS, SR
Metabolite B	H	Glucuronide	RR,SS
Metabolite C	Glucuronide	H	Mixture

The same three metabolites were isolated from pig urine and were all identified by FAB mass spectroscopy and NMR to be monoglucuronides of RACT. Using NMR decoupling experiments and comparison of NMR shift data of the aromatic protons with RACT and deshydroxy RACT, the glucuronide was identified to be

attached to Ring B in Metabolites A and B and to Ring A in Metabolite C. The difference between Metabolites A and B is presumed to be that of stereoisomerism, i.e. one is the RR,SS diasteromeric pair and the other is the RS, SR diasteromeric pair. Metabolite C data indicates that it too is a stereochemical mixture.

Quantitation of the HPLC data showed that in the liver Metabolites A and B constituted 30.9% and Metabolite C 4.6% of the extractable radioactive residues. The kidney contained 31.0% Metabolites A and B and 25.9% Metabolite C. The chromatographic profiles of the liver extracts on silica gel columns and HPLC of XAD-2 fractions were the same for livers from double-ring labeled and single-ring labeled ^{14}C- RACT studies. Ring separation, therefore, does not occur, and the ^{14}C- residue concentrations in edible tissues are true indications of all RACT related residue in all studies.

VI.C.2. ^{14}C- Ractopamine Hydrochloride Balance-Excretion Study in Swine. Study ABC-0330.

INVESTIGATORS: J. E. Dalidowicz, Ph.D.,
T. D. Thomson, Ph.D., and R. J. Herberg, M.S.
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The purpose of this study was to determine the rate, route, and completeness of excretion of a single oral dose of ^{14}C-RACT in swine. A lot of 97+% pure ^{14}C-RACT, uniformly labeled in Ring A, was mixed with unlabeled RACT to make the test article for these studies. Three crossbred swine (two barrows and one gilt) weighing approximately 45 kg were each fed 1.0 kg of feed containing 20 ppm of unlabeled RACT twice daily for five days. At the end of the pre-dosing period, each pig received a one time dose of 40 mg of ^{14}C- RACT (0.5 µCi/mg) incorporated into control feed. The swine then continued to receive 1.0 kg of ration containing 20 ppm of unlabeled RACT twice daily until termination of the experiment.

The entire urinary and fecal output of each animal was collected at 24-hour intervals. Samples were analyzed for radioactivity.

During the seven-day collection period, the three animals excreted an average of 96.5% of the administered ^{14}C- RACT. Of this, 88.1% was found in urine and 8.4% in feces. Ninety-five and four tenths percent of the ^{14}C- RACT dose was excreted in the first three days with 84.7% eliminated during the first day. It was concluded that swine receiving RACT in the diet eliminate nearly 85% of the administered dose during the first day and over 95% within three days. Urine excretion was the predominant route.

VI.C.3. ^{14}C-Ractopamine Hydrochloride Steady-State Residue Study with Swine Fed 30 ppm, or 1.5X the Highest Anticipated Dose. Study ABC-0273.

INVESTIGATORS: J. E. Dalidowicz, Ph.D.,
T. D. Thomson, Ph.D., and R. J. Herberg, M.S.
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The length of time required for radioactive residues to reach steady state in swine tissues was determined. Three sets of three swine were fed 30 ppm (1.5 times the highest anticipated dose) of ^{14}C- RACT for four, seven, or ten days. Approximately 12 hours (practical zero withdrawal) after the last feeding, animals in each group were euthanized and total radioactive residues were measured in muscle, fat, liver, and kidney. No detectable residues were measured in the fat of the three groups of animals. The mean levels of total radioactivity, expressed as ppm of RACT equivalents, in each group ranged from 0.254-0.424 ppm for livers, 0.466 - 0.655 ppm for kidneys, and 0.019-0.024 ppm for muscle. Statistical analysis of the residues from the three groups of animals showed that total residues reached steady state levels four days after initiating RACT feeding. The mean levels of non-extractable residues were also measured in study ABC-0273. Like the total radioactive residue, non-extractable residues reached steady state after four days of feeding RACT. Mean levels of non-extractable radioactivity ranged from 26.0 - 29.1% of the total radioactive residue in liver, and 14.6 - 15.9% of the total residue in kidney.

	Total Radioactive Residues (ppm) Days Fed		
Tissue	4	7	10
Muscle	0.021	0.019	0.024
Kidney	0.518	0.466	0.655
Liver	0.364	0.254	0.424
Fat	NDR	NDR	NDR

VI.C.4. The Depletion of ^{14}C- Ractopamine HCl Residues in Swine During Short Withdrawal Periods. Study ABC-0283.

INVESTIGATORS: J. E. Dalidowicz, Ph.D.,
T. D. Thomson, Ph.D., and R. J. Herberg, M.S.
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Three barrows and three gilts received 14C-RACT at 30 ppm in the feed for four days. Groups of two swine of mixed sex were then slaughtered after zero-, one-,

and two-day withdrawal periods and the 14C-residue concentration determined in the kidneys, liver, muscle, and fat.

The average radioactivity found in each of the four tissues at the different withdrawal times, calculated as net ppm of RACT is summarized in the following table (NDR = no detectable residues):

	Total Radioactive Residues (ppm)		
	Withdrawal Time		
Tissue	Zero	One-Day	Two-Day
Muscle	0.01	0.01	NDR
Kidney	0.46	0.13	0.06
Liver	0.31	0.17	0.07
Fat	0.01	0.01	<0.01

VI.C.5. ^{14}C-EL-737 Tissue Depletion Study in Swine. Study ABC-0291.

INVESTIGATORS: J. E. Dalidowicz, Ph.D.,
T. D. Thomson, Ph.D., and R. J. Herberg, M.S.
Lilly Research Laboratories
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Six barrows and six gilts received ^{14}C-EL-737 (RACT) at 30 ppm in the feed for four days. The dose was one and one half times the highest anticipated use level (20 ppm). Groups of three swine of mixed sex were then slaughtered after zero-, two-, four-, and seven-day withdrawal period and the ^{14}C-residue concentration determined in the kidneys, liver, muscle, and fat.

The average radioactivity found in the four tissues at the different withdrawal times, calculated as net ppm of RACT, is summarized in the following table:

	Total Radioactive Residues (ppm)			
	Withdrawal Time			
Tissue	Zero	Two-Day	Four-Day	Seven-Day
Muscle	0.02	0	0	0
Kidney	0.60	0.06	0.03	0.02
Liver	0.42	0.10	0.05	0.06
Fat	0.02	0	0	0

VI.C.6. ^{14}C-Ractopamine Hydrochloride Swine Tissue Residue Study. Study ABC-0368.

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The relationship between parent RACT (marker residue) and the total radioactive residue was established in target tissues so that the total residue in a tissue could be calculated by measuring only the marker residue in swine fed non-radioactive RACT under field conditions. Three barrows and three gilts (45 kg) were fed a ration containing 30 ppm (1.5 x the highest anticipated use) of ^{14}C- RACT for four consecutive days. Twelve hours after the last feeding (a practical zero-time withdrawal period) each animal was euthanized and both the total radioactive residue in liver and kidney and the amount of residue present as parent compound was determined. The relationship between parent RACT and total residue was calculated as shown below.

Tissue	Concentration (ppm)		%
	^{14}C Residues	Ractopamine HCl	Ractopamine HCl
Kidney	0.405	0.094	23.4
Liver	0.410	0.111	27.2

Summary Of The Zero-day Withdrawal Data From The Four ^{14}C- Residue Studies

Tissue	STC*	Mean Total Radioactive Residue (ppm) Zero-Day Withdrawal			
		ABC-0273^	ABC-0283	ABC-0291	ABC-0368
Liver	0.75	0.347	0.31	0.42	0.410
Kidney	1.50	0.546	0.46	0.60	0.405
Muscle	0.25	0.021	0.01	0.02	ND**
Fat	1.50	NDR#	0.01	0.02	ND

* Safe Tissue Concentration
^ All those animals receiving ractopamine >/= 4 days (steady state)
# No Detectable Residues
** Not Determined

VI.D. Comparative Metabolism

VI.D.1. Comparative Metabolism of ^{14}C -Ractopamine in Swine, Dogs, and Rats. Studies ABC-0301, ABC-0285, and ABC-0369.

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The purpose of this study was to compare the metabolism of RACT in swine, dogs, and rats. Radiochemically equivalent amounts of two lots of 98+% pure ^{14}C-RACT, one uniformly labeled in Ring A, the other uniformly labeled in Ring B, were mixed with unlabeled RACT to make the test article for these studies.

Six crossbred pigs (three barrows and three gilts) weighing approximately 45 kg each were fed the test article at 30 ppm in the diet for four days. The pigs were killed 12 hours after the last dose. Two adult beagle dogs (one male and one female) weighing 7-8 kg were dosed by gavage with the test article at 0.5 mg/kg (0.25 mg/ml H2O) three times daily for four days and once on the fifth day. The dogs were killed three hours after the last dose. Twelve male and twelve female Fisher 344/NHSD rats approximately 8-9 weeks of age and weighing

approximately 120-200 g, were dosed with the test article by gavage at 2 mg/kg/day (10 ml/kg) for five days. The rats were killed three hours after the last dose. Liver and kidney tissues from the pigs, dogs, and rats were analyzed by HPLC and scintillation counting. The mean amounts in ppm of RACT and its metabolites (calculated as RACT) in kidney and liver tissues were:

	Liver			Kidney		
	Swine	Dog	Rat	Swine	Dog	Rat
Ractopamine HCl	0.12	0.59	0.40	0.10	0.50	0.33
Metabolite A	0.03	0.46	0.17	0.05	0.18	0.52
Metabolite B	0.04	0.77	0.15	0.06	0.27	0.57
Metabolite C	0.02	1.76	0.10	0.09	0.63	0.08

It was concluded that the dogs and rats used in the toxicological studies were exposed to the same metabolites as those found in the edible tissues of pigs.

VI.E. Withdrawal Time and Tolerance

Data from four residue studies utilizing ^{14}C- RACT were analyzed to determine the total residues in edible tissues of swine at zero days of withdrawal of the drug from the diet. All of these studies demonstrate that the total residues in swine fed a diet containing 30 ppm of RACT do not exceed the safe tissue concentrations. These studies therefore support the approval of the use RACT in swine rations at up to 20 ppm with a zero-day withdrawal.

Although the application included sufficient data to support a zero withdrawal period the Agency has determined it appropriate to set a tissue residue tolerance (Rm) for monitoring purposes. After examining the data from studies submitted for characterizing the metabolites and residues of RACT in the marker tissue (liver) at zero withdrawal, it was decided that a conservative value of 20% for the amount of marker residue (RACT parent) in the total would be used for setting the tolerance. The safe tissue concentration for total residues in liver is 0.75 ppm, therefore the Rm for RACT parent in liver is set at 0.15 ppm (20% of 0.75). In addition, a tolerance of 0.05 ppm is established for RACT parent in swine muscle by extrapolating the 20% value to muscle (i.e., 20% of 0.25 ppm).

VI.F. Regulatory Method

Although the application included sufficient data to support a zero withdrawal period, normally making an official regulatory method unnecessary, the Agency has determined it appropriate to evaluate a regulatory method for tissue residues. Analytical methods suitable for use by regulatory authorities for the detection and confirmation of RACT residues in swine tissues have therefore been developed and validated.

The determinative assay for measuring RACT residues in treated swine consists of extraction of the parent drug from liver or muscle, and measurement of the parent drug in the extract by high performance liquid chromatography (HPLC).

Ground swine liver or muscle is homogenized with methanol (MeOH) and centrifuged to separate the solids. A portion of the MeOH extract is evaporated to dryness and reconstituted in borate buffer and partitioned with ethyl acetate. RACT is further isolated from the matrix by passing the ethyl acetate extract through an Alumina A solid phase extraction cartridge. RACT is eluted from the cartridge with MeOH. The MeOH extract is evaporated to dryness and reconstituted in two percent aqueous acetic acid for HPLC analysis. HPLC analysis is carried out using a reversed-phase octadecylsilyl stationary phase with fluorescence detection at 305 nm (excitation at 226 nm).

The limit of quantification of the method is 2 ppb.

To confirm the identity of RACT in tissues, ground swine liver or muscle is homogenized with methanol (MeOH) and centrifuged to separate the solids. A portion of the MeOH extract is evaporated to dryness, reconstituted in borate buffer, and partitioned with ethyl acetate. RACT is further isolated from the matrix by passing the ethyl acetate extract through an Alumina A solid phase extraction cartridge. RACT is eluted from the cartridge with MeOH. The MeOH extract is evaporated to dryness and reconstituted in ammonium acetate buffer for analysis. Samples are subjected to analysis by reversed-phase high performance liquid chromatography/ionspray mass spectrometry (HPLC/ISP-MS). The mass spectrometer operating parameters are set up to monitor three structurally indicating fragment ions unique to RACT.

Method trials of the determinative and confirmatory assays for swine liver were satisfactorily completed by a combination of Food and Drug Administration (FDA), U.S. Department of Agriculture, and private laboratories.

The validated regulatory analytical methods for detection and confirmation of residues of ractopamine in liver and muscle are on file at Center for Veterinary Medicine's Document Control Unit (HFV-199), FDA, 7500 Standish Place, Rockville, MD 20855.

VI.G. User Safety Concerns

RACT is pharmacologically active as a partial beta adrenergic agonist. Acute and chronic exposures of mammals to RACT at sufficiently high levels by the oral, inhalation, or intravenous injection routes results in the signs expected from this class of compounds: increased heart rate, increased blood and pulse pressure, peripheral dilation of blood vessels, and increased cardiac output. Monkeys exposed for four hours per day for 8 days to airborne levels of RACT of 0.38 mg/m^3 or greater experienced increased heart rates while 15 minute inhalation

exposures resulted in increased heart rate at concentrations of 13.9 mg/m^3 and greater (2.4 mg/m^3 was a no-effect-level). The Paylean®Type A Medicated Article was formulated with an oil coating to minimize the inhalation exposure during use of the product. To estimate the potential for feed mill operators to experience significant inhalation exposures to ractopamine during handling and mixing operations, an exposure monitoring study was conducted. Under the conservative conditions of the on-site study, measurements from the mill operators' personal air samplers demonstrated mean exposure values of < 0.001 mg/m^3 during short term weighing and bagging operations and long term operations in the mill resulted in exposure values of <0.0002 mg/m^3.

RACT is an eye irritant in rabbits and at very high exposure levels (5,000 mg/kg) is a slight skin irritant. In Guinea pigs, RACT was a contact sensitizer. In rodents there were no effects on mating performance or fertility, but increased mortality, growth retardation, and structural abnormalities were seen in offspring where doses were high enough to be maternally toxic.

User safety concerns associated with effects of accidental inhalation or direct contact have been satisfactorily addressed by formulating the product to minimize dust generation and by establishing label warnings. In addition, a toll-free telephone number will be available on the label to inform users of where they can obtain additional information concerning user safety, request an MSDS, and to report adverse effects.

VII. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that ractopamine hydrochloride (Paylean® TYPE A MEDICATED ARTICLE) when administered in the diet at 4.5 to 18 grams/ton (5 to 20 ppm) to finishing pigs from 150 to 240 lb and fed a complete ration containing at least 16% crude protein is safe and effective for the improvement of feed efficiency and increased carcass leanness. Further, when ractopamine hydrochloride is administered in the diet at 4.5 grams/ton (5 ppm) to finishing pigs from 150 to 240 lb and fed a complete ration containing at least a 16% crude protein is safe and effective for the increase of average daily body weight gain, the improvement of feed efficiency, and increased carcass leanness.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions for use by the layman have been provided. Historically, the industry is familiar with the handling and mixing of Type A medicated articles into Type B and C medicated feeds. Ractopamine hydrochloride is not a controlled substance. Thus, labeling is adequate for the intended use.

Ractopamine hydrochloride was not found to cause cancer in rats and mice chronically exposed to high dietary levels of the drug. Based on a battery of toxicology tests, an acceptable daily intake (ADI) of 1.25 μg/kg/day has been established for ractopamine hydrochloride in the human diet. A marker residue tolerance is established for ractopamine parent of 0.15 ppm in liver, the target tissue, for monitoring purposes. A muscle tolerance of 0.05 ppm has been

established for residues of ractopamine parent in swine muscle. Residues of ractopamine in swine muscle are not indicative of the safety of residues in other edible tissue. The total residue data showed that the mean concentrations of total ractopamine hydrochloride residues at 12 hours after feed withdrawal were well below the permitted safe concentration in the edible tissues of finishing pigs. Husbandry practices for pigs are such that they will not enter the human food chain until 12 or more hours after the pigs are removed from feed. Therefore, a zero-day withdrawal period will be established for this use of ractopamine hydrochloride in finishing pigs.

Analytical methods suitable for use by regulatory authorities for the detection and confirmation of ractopamine hydrochloride residues in swine tissues have been developed and validated.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment, which may be seen in the Dockets Management Branch (HFV-305), 5630 Fishers Lane, rm. 1061, Rockville, Maryland 20852.

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for 5 years of marketing exclusivity beginning on the date of approval because no active ingredient (including any ester or salt of the active ingredient) has been approved in any other application.

Ractopamine hydrochloride is under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
4,690,951	September 1, 2004
4,734,437	September 1, 2004

VIII. APPROVED PRODUCT LABELING:

A. PAYLEAN® TYPE A MEDICATED ARTICLE BAG LABEL
B. PAYLEAN® TYPE B MEDICATED FEED BLUEBIRD BAG/TAG LABEL
C. PAYLEAN® TYPE C MEDICATED FEED BLUEBIRD BAG/TAG LABELS